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THE INTRADERMAL TEST FOR  
TUBERCULOSIS IN CATTLE  
AND HOGS

BY  
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ASSISTED BY  
RALPH M. BELL

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## FOREWORD

In all ages, trees and grasses have been the most important products of the soil. The efficient, economic utilization of grass and similar forage, which can be accomplished only by the use of domestic animals, is one of the world's great problems. It is a basic problem in California. Animals are necessary to utilize the grass on its vast ranges, including valleys, foothills, and mountains, and also to utilize one of the most important products of irrigation—alfalfa. The production of butter fat is almost an economic necessity wherever alfalfa is extensively raised. Domestic animals, however, do more than convert into edible form the products of the soil. They help to maintain its fertility: first, by returning to it essential fertilizing ingredients and, second, by permitting a more diversified system of agriculture.

Obviously one of the most important factors in the efficiency of this living machine is its health. If animals when grown are unfit for food, in so far as this occurs it becomes a tax upon every pound of wholesome beef. If the average usefulness of a cow extends only through three periods of lactation, as is claimed to be the fact in some milch dairies, the cost of producing every pound of butter fat and every bottle of milk is thereby increased.

The most disastrous disease among cattle is tuberculosis. It is a menace that no stockman or dairyman can afford to ignore. Its control is a problem that every state must seek to solve as surely as it must endeavor to develop an abundant food supply.

Of the eight thousand head of cattle examined under the direction of the veterinary division of this Station, approximately twenty-five hundred have reacted to the tuberculin test. The loss from this disease in California alone is estimated at over half a million dollars per year. The loss appears to be increasing. Reports of meat inspectors in San Francisco and Los Angeles indicate that the proportion of cattle from certain ranges, which are affected with tuberculosis, has increased from 1 to 5 per cent in five years.

The Agricultural Experiment Station has resolutely set itself to study this problem with all the resources at its command. It is going to be a long, hard road to travel. A thorough study of the conditions under which this insidious disease is spread among domestic animals must be made. Then a sane, rational, comprehensive system of public control must be organized. All evidence points to the fact that it must be on lines not heretofore generally attempted in this country.

Up to the present time the most efficient weapon with which to combat tuberculosis in cattle has been the tuberculin test. A description of the usual method of performing this test was published by this Station in Bulletin No. 199, entitled "Bovine Tuberculosis," which may be obtained free upon application. This method, however, of performing the tuberculin test by injecting the tuberculin subcutaneously has not proved effective in all cases. It cannot be applied satisfactorily to young calves or to wild range cattle, while during the hot season in some of the interior valleys the test has been unsatisfactory even when applied to docile dairy cows. A recent modification of the tuberculin test, called the *intradermal method*, is especially adapted to these conditions. This bulletin deals with the best technic and the proper strength of the tuberculin to be used in this new method, as well as its accuracy as compared with the usual subcutaneous method. The intradermal method is believed to constitute an important step in the control of tuberculosis in both cattle and hogs. However, it should be clearly understood that a method of discovering the disease, which any tuberculin test is, is not, in itself, a method of controlling it. When the proper knowledge has been obtained of the conditions under which the disease spreads, a system of husbandry must be developed which will ensure the maintenance of healthy animals.

THOMAS F. HUNT,

Director.

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## GENERAL CONCLUSIONS REGARDING THE INTRADERMAL METHOD OF TUBERCULIN TESTING CATTLE

The intradermal method of tuberculin testing cattle as first applied by the French investigators Moussu and Mantoux<sup>37</sup> and as used under California conditions by Ward,<sup>50</sup> Baker, Longley<sup>23</sup> and others has many advantages over the usual method. Since the reaction to tuberculin when injected intradermally depends upon a swelling at the point of injection and not on a rise of temperature, it is to be preferred to the subcutaneous under all conditions that are liable to modify the tuberculin temperature curve, such as unusual surroundings, very hot weather, or fatigue from a long journey. It can be satisfactorily applied to young calves and wild range cattle and is especially useful in testing cattle during the hot season in the interior valleys of this



state under conditions that render the subcutaneous method of testing unsatisfactory. It excels the subcutaneous method in economy of time, labor and materials.

From our observations in applying 4926 intradermal tests, including retests, to 4001 head of cattle, 1614 of which reacted, the results being checked by 1000 subcutaneous tests and 341 autopsies we conclude that under average dairy conditions the intradermal equals the subcutaneous method in accuracy, provided a 5 per cent, or stronger, solution of alcoholic precipitated tuberculin is used and the test is performed by an experienced operator. We do not advocate the substitution of the intradermal for the subcutaneous method by a veterinarian until he has become skilled in its use by practice and observation. The practitioner can easily accomplish this in the routine of his work by applying the two tests simultaneously. Such a procedure cannot injure the accepted subcutaneous method in any way, although it may modify the local intradermal swelling to some extent.

Tuberculous cattle usually react thermally, as well as locally to intradermal injections, even when very small doses of tuberculin (1 to 10 milligrams) are used. If temperatures are taken at two-hour intervals from the eighth to the twentieth hours following the intradermal injection it will be found that most of the cattle which react locally will also react thermally.

Since neither the intradermal, the subcutaneous, nor the ophthalmic method will detect every case of tuberculosis when applied independently, the maximum number of reactors can only be detected by applying the combined tests. This may be done by applying the intradermal and subcutaneous tests simultaneously or by administering the intradermal test alone and retesting all of the non-reactors after an interval of at least seven days. The ophthalmic test may also be advantageously combined with either or both of these tests. The ophthalmic test alone is unsuitable for use under the average California dairy conditions, except when only a few animals are to be tested and they can be kept confined under shelter. For official tuberculin testing where a high degree of efficiency is necessary a combination of the three tests would be desirable. The intradermal method is especially adapted to the testing of swine.

## RESULTS OBTAINED WITH TUBERCULIN IN TESTING CATTLE AND HOGS BY THE INTRADERMAL AND OTHER METHODS

BY

CLARENCE M. HARING

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The intradermal or intracutaneous\* method of applying the tuberculin test to cattle is now regularly used by several veterinary practitioners in California, and reports from other states and from foreign countries indicate that the method is rapidly gaining favor among veterinarians elsewhere. The test possesses such advantages in economy of time and materials that it has been widely accepted by practitioners as a substitute for the subcutaneous method, without waiting to determine whether it is sufficiently accurate to warrant such an acceptance. The data concerning its accuracy, as compared with that of the usual subcutaneous method, are not sufficient, however, to convince many state officials of the advisability of accepting the intradermal test as a substitute. Another new method of tuberculin testing, called the ophthalmic, conjunctival or eye test, is used in some sections. The ophthalmic method, although not popular in this country, is preferred by some veterinarians. In regard to the acceptance of these methods for the admission of cattle into states requiring a tuberculin test certificate, the following information is of interest. Statements have been received by the writers from the livestock sanitary authorities of thirty-nine states. In Delaware and California the intradermal test is accepted, if performed by an approved veterinarian. The State Veterinarian of Pennsylvania writes that he would not recommend the livestock sanitary board to recognize the method indiscriminately except under certain conditions when the intradermal or ophthalmic tests were made by an unusually well-qualified veterinarian and a certificate for physical examination accompanied a report of the test. The State Veterinarian of Missouri writes: "We are using the intradermal test exclusively in our state

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\* The terms intradermal and intracutaneous are synonyms. Custom renders the word intradermal preferable, when mentioned in connection with the testing of cattle.



work, but for interstate shipments we are using the thermal method, for the reason that the intradermal leaves no record. Therefore, until such time as an agreement can be made between the different states and there is a better system for the control of the veterinarians, it will be necessary for us to require the thermal test and a record of the same filed in this office immediately after the inspection is made. We have the greatest confidence in the intradermal test and would not hesitate to accept shipments where it has been applied, except for the fact that only a very small per cent of the veterinarians doing interstate work are familiar with the intradermal test. Therefore, I see no possibility of our accepting the intradermal test in the near future." Most of the state authorities agree that they cannot as yet officially accept either the intradermal or ophthalmic method. The consensus of opinion seems to be that these tests are fairly satisfactory when administered by careful and experienced operators, but that it is unsafe to accept such tests from veterinarians indiscriminately, until these methods have come into more general use and have been accepted by the United States Bureau of Animal Industry.

#### LOCAL TUBERCULIN REACTIONS AND THEIR USE FOR DIAGNOSIS IN HUMAN AND VETERINARY PRACTICE

In human practice\* the intradermal method is used to some extent, but many physicians consider this method too delicate for routine diagnosis in man. Their objection is that the test is so searching in its scrutiny that it causes reactions in latent cases which would probably never develop active tuberculosis.

In veterinary practice, however, this "searching scrutiny" is usually desired and from data already available there is reason to believe that this test *when combined with observations concerning the*

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\* The tissues of tuberculous persons and animals are sensitive to tuberculin. This sensitiveness is most marked in the tissues that are actually invaded by the germs, but it also exists to some extent in the other tissues of the body which contain blood vessels. The living tissues of individuals who have not been infected with tuberculosis show no sensitiveness to tuberculin.

Attention was first called to the possibilities of the intradermal injection of tuberculin as a means of diagnosis by Mendel, who suggested that the procedure followed in the intradermal method of injecting for anesthesia would be a delicate and satisfactory means of diagnosis. The suggestion was soon put into practice by Mantoux and others. According to Hamman and Wolman,<sup>12</sup> the intradermal method when applied to man has no equal as a means of estimating the degree of tuberculin hyper-sensitiveness. They conclude from a study of the records of a large number of tests made by the various methods of tuberculin testing that the intradermal and subcutaneous local tests are the most delicate tests for man. They reveal practically the full percentage of tuberculosis infected individuals. In the order of their sensitiveness the tests

*thermal reaction furnishes a more accurate means of diagnosis than the usual subcutaneous method of testing cattle*, in which no attention is paid to the local reaction and no attempt is made to select and prepare the field of injection and to inject in such a way that if the local reaction occurs it will be easily detected. Many veterinarians in making the subcutaneous test shave the neck at the point where the subcutaneous injection is to be made, in order to more easily observe the local reaction which frequently occurs. Corbett and Griffiths<sup>6</sup> state that the local reactions resulting from subcutaneous injections in the neck were usually very slight and of no special value. However, we have found that in interpreting doubtful thermal reactions it is of assistance to examine the site of injection for local swellings. One of us (Bell) has found it of value, in testing by the subcutaneous method, to inject the tuberculin through the skin of the hindquarter over the semimembranosus muscle. He finds that the local reactions to the subcutaneous injections are more clearly defined than in the loose tissues of the neck. The intradermal method excels the subcutaneous in the size and definiteness of its local reactions.

#### TECHNIC OF THE INTRADERMAL METHOD

The manner in which the intradermal differs from the usual subcutaneous test is that the tuberculin is injected in  $\frac{1}{20}$  to  $\frac{1}{4}$  cc. amounts into the deeper layers of the skin, or into the subdermal tissue immediately adjacent, instead of injecting a large quantity (1 to 4 cc.) deeply under the skin. The most suitable place to make this injection is in one of the folds of the skin on the under side of the base of the tail. We have used the skin of the neck in some of our tests, but find the subcaudal fold more convenient. The skin

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for tuberculosis in man arrange themselves according to Hamman and Wolman<sup>11</sup> as follows:

- Intracutaneous test (Intradermal)
- Subcutaneous-local test (Strichreaction)
- Cutaneous test (von Pirquet)
- Subcutaneous test (Koch)
- Percutaneous test (Moro inoculation)
- Conjunctival test (Calmette ophthalmic).

As a means of diagnosis of tuberculosis in man, the first two tests are said by these writers to be undesirable for the very reason that they detect even the latent cases of infection. They state that in adult life and even in late childhood the subcutaneous local reaction is of absolutely no diagnostic value, since only an occasional individual will escape its searching scrutiny. What little diagnostic value it has is restricted to the first two years of life and even at that age it has no marked value above the cutaneous test for the reason that these tests have shown the astonishing extent to which man is tuberculosis-infected and at what an early age the infection occurs.

here is free from hair and is soft and pliable. A hypodermic syringe with a short needle point should be used. Such a syringe should have a 25 or 26 gage needle with a point  $\frac{3}{16}$  to  $\frac{1}{4}$  of an inch in length, similar to those used by dentists for injecting local anaesthetics (see plate 1, figures 1 and 2).

The subcaudal fold is grasped between the thumb and the first two fingers of the left hand and the needle inserted horizontally into the thickness of the skin grasped between the thumb and finger (see plate 2, figure 1). The  $\frac{1}{10}$  or  $\frac{1}{5}$  cc. dose, if properly placed, can be felt in the layers of the skin as it is expelled from the syringe, where it remains as a small lump in the skin after the needle has been removed. In our first tests we made the mistake of trying to inject as near the surface of the skin as possible. It is difficult to inject into the layers of the epidermis and an injection into this part of the skin is of little diagnostic value. In case the needle is of the proper length, namely, one-quarter of an inch, there is little danger of going completely through the skin. When the proper point in the subcaudal fold is selected, it makes little difference whether the point of the needle is in the derma or in the subdermal connective tissue. With the proper syringe an expert operator can inject in the dark as accurately as in a good light. We have found that characteristic reactions occur with the injections from a long needle, placed completely through and beneath the skin layers of the subcaudal fold. It is well to inject at a point on the fold about two and one-half or three inches down the tail from the anus, since elsewhere reactions are not so easily perceived and at this point the bone and solid tissue of the tail form a background which renders the local reactions more prominent than those of the skin of the neck.

After successfully using the intradermal method on fifteen hundred head of cattle, Longley<sup>23</sup> has expressed the opinion that it is impossible to inject into the skin proper, but that the dose goes into the subdermal connective tissue. From experiments which we have made on the hides of recently killed cattle, we think he must have meant the epidermis. When a one-quarter inch needle is inserted slightly horizontally into the subcaudal fold it rarely penetrates deeper than the lower layers of the derma or true skin.

In injecting cattle that are secured in stanchions, a convenient way is to stand by the side of the cow, as shown in plate 2, figure 1, pressing the knee into the animal's flank and grasping the loose folds of skin from under the tail without raising the tail. Some operators, when injecting, prefer to stand directly behind the cow, as shown in plate 2, figure 2, but this method is impracticable except with very

docile cattle or when an assistant is available to steady the animal and keep her from moving from side to side.

We deem it impracticable to disinfect or otherwise prepare the subcaudal fold for the intradermal injection, except when it is soiled. In such cases, wipe the fold clean with a rag, apply 50 per cent alcohol with absorbent cotton and wipe with dry cotton. Before injecting each animal, the syringe needle should be dipped in strong disinfectant, and wiped dry with sterile absorbent cotton. After dipping the needle in an irritating disinfectant, take pains to wipe it off before inserting it, for the disinfectant may cause irritation and produce a swelling which might be mistaken for a reaction. The application of strong disinfectants at the point of intradermal injection should be avoided. In several instances we have observed swellings, varying in size from a hazelnut to a hen's egg, on the subcaudal fold as a result of the application of strong lysol solution. These might have been mistaken for reactions, but for the fact that the cattle were not injected with tuberculin (see plate 6, figure 1).

#### KIND AND STRENGTH OF TUBERCULIN FOR INTRADERMAL USE

From experiments which we have conducted during the past year, we believe that a strong solution of *alcoholic precipitated tuberculin*\* is most suitable for use with the intradermal test in cattle. In order

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\* Laboratories vary considerably in their methods of preparing precipitated tuberculin. Most of the tuberculin which we have used has been prepared as follows: To twenty volumes of absolute alcohol add very slowly one volume of Koch's Old Tuberculin concentrated. Stir thoroughly and set aside for twenty-four hours. At the end of this time a flocculent deposit will be seen in the bottom of the vessel. The supernatant fluid is decanted and the partially dried precipitate redissolved in one volume of 1 per cent sodium chloride solution and the tuberculin reprecipitated by adding this solution to twenty volumes of absolute alcohol. The supernatant fluid is decanted and the precipitate washed by adding it to nine volumes of absolute alcohol. The precipitate is then placed on clay plates, put in a dessicator and, under vacuum, dried over sulphuric acid. A brownish powder can be scraped from the plate. This is the *precipitated tuberculin*; also known as dry tuberculin or tuberculin siccum. This tuberculin is said to be soluble in the proportion of one part by weight to ten of water, but higher concentrations can sometimes be obtained. We have been able to make 50 per cent solutions. According to Brieger and Proskauer,<sup>8</sup> one-tenth of a gram of this precipitated tuberculin produces the same effect as five-tenths of a gram of crude tuberculin (Koch's Old). According to Hamman and Wolman this kind of tuberculin seems to be more toxic to animals than Koch's Old Tuberculin.

This may be dispensed in powder form to be made into a solution when needed. Most laboratories, however, prepare the solution before shipment, a diluent of  $\frac{1}{2}$  per cent of trikresol being added and the solution sterilized by steaming at 60° C. for thirty minutes.

The concentrated tuberculin used in preparing the precipitated should be made from selected cultures. The culture media used should be prepared according to standard methods, using veal broth and not that prepared from commercial extract of beef. We have found some of the precipitated tuberculin on the market to be of low potency.



to obtain a distinct and well-marked subcaudal reaction this purified tuberculin should be used in at least a 5 per cent solution (50 milligrams per cc. of physiological salt solution) administering doses of between  $\frac{1}{10}$  and  $\frac{1}{5}$  cc. The injection of ten times this dose ( $\frac{1}{2}$  cc. of 25 per cent solution) subcaudally leaves no perceptible swelling in non-tuberculous animals after the forty-eighth hour while in tuberculous cattle a swelling the size of an egg usually results. Vallee<sup>48</sup> and his co-workers endeavored to obtain the subcaudal reaction with the minimum dose, but in order to obtain the maximum number of reactions in tuberculous cattle, we believe it is desirable to use a stronger solution than they suggest. This point is discussed later in connection with tuberculin manufactured by various firms. Ten per cent solutions of potent precipitated tuberculin (100 milligrams per cc. of physiological salt solution) will usually produce pronounced local and thermal reactions in tuberculous cattle, when administered intradermally.

Koch's Old Tuberculin in 10 per cent solution has also given good results as can be seen by an inspection of Tables 1 and 2. The tuberculin distributed by the Bureau of Animal Industry for official use is equivalent to 10 per cent Koch's Old Tuberculin and for the practitioner who can obtain it this tuberculin will doubtless prove more satisfactory than the solutions of tuberculin sold for intradermal use by biological houses.

We have also obtained excellent results in both the intradermal and ophthalmic work with *phymatin*, a tuberculin of unknown content manufactured in Germany.

Hutyra and Marek<sup>15</sup> have suggested that the tuberculins which contain ground or pulverized tubercle bacteria will probably be found to be most potent in producing local reactions. In view of this we tried two such preparations which, by courtesy of the Cutter Laboratory, were manufactured especially for these experiments. One of these consisted of a glycerin free suspension of finely ground tubercle bacilli. The other consisted of a similar suspension of whole tubercle bacilli killed by steaming for two hours at 60° C. The emulsions contained a solid content of from 10 to 20 milligrams tubercle bacilli per cc. In tuberculous animals no local or thermal reactions resulted from the intradermal injection of  $\frac{1}{10}$  cc. doses of these suspensions.

*Precautions Against the Deterioration of Precipitated Tuberculin.*—Meyer<sup>33</sup> has called attention to the fact that aqueous solutions of alcoholic precipitated mallein disintegrate rapidly, due in part to the absence of preservative and in part to changes in toxin molecules. Therefore, the powder form alone can be kept in stock and the test

solution should always be made shortly before use. It is probable that this holds true for solutions of precipitated tuberculin as well. As a diluent for the dry tuberculin we have used  $\frac{1}{4}$  per cent solutions of trikresol in physiological salt solution and, if the dilution is prepared aseptically, we have found that the tuberculin dilution will remain sterile and potent for months. The use of trikresol or phenol in  $\frac{1}{4}$  to  $\frac{1}{2}$  per cent solutions for the intradermal test does not appear to produce a perceptible local irritation or be objectionable in any way. On account of the possibility of molecular disintegration, however, we advise that the test solution be prepared shortly before use. It is convenient to keep on hand a stock of sterile 2 cc. amber glass bottles, each bottle containing one-tenth of a gram of dry alcoholic precipitated tuberculin. By adding to one of these 2 cc. of sterile physiological salt solution, ten doses of 5 per cent tuberculin solution can be prepared. As the tuberculin powder goes into solution very slowly, the diluent should be added at least two hours before the injection is to be made. On account of the detrimental effect of light on such solutions they should be kept in amber bottles and protected from direct sunlight as much as possible.

*Lack of Uniformity in Tuberculin.*—In the United States there is a great lack of uniformity regarding the tuberculin used. We have purchased “intradermal” tuberculin from six different firms, and in only two instances has the product been the same, as is shown by the following:

*Firm A* recommends for the intradermal test in cattle a tuberculin bearing the label “Tuberculin O. T. (Original) Serial Dilution No. 5, 1 cc. contains 100 mg.” This they state is prepared by diluting one part of Koch’s Old Tuberculin with nine parts of physiological salt solution containing  $\frac{1}{4}$  per cent trikresol.

Recently this firm has placed on the market “Intradermal Tuberculin, Veterinary,” which is essentially the same as the above in strength, although in its preparation it is not deemed necessary to concentrate the original culture down to the full equivalent of Koch’s old tuberculin before diluting it back to the strength of dilution No. 5.

On request they will also furnish for the intradermal test dilutions of alcoholic precipitated tuberculin, glycerin free, in any dilution desired up to 10 per cent solution.

*Firm B* recommends for the intradermal test of cattle a tuberculin bearing the label “Solution Purified Tuberculin for Intradermal Test. Each cc. contains 50 milligrams purified and precipitated Tuberculin in sterilized normal salt solution. Dose—1 to 5 minims.” On request, this firm will also furnish for the intradermal test stronger solutions of alcoholic precipitated tuberculin.



*Firm C* recommends for the intradermal test in cattle "Ordinary Veterinary Tuberculin." This is presumably similar to that distributed by the Bureau of Animal Industry for the use of state and municipal officials.

*Firm D* states that they do not dispense any especially prepared tuberculin for the intradermal test, but recommends their "concentrated tuberculin." The label on this tuberculin reads as follows: "Tuberculin, Veterinary, ready for use." This firm advises us by letter that this concentrated tuberculin is seven times the strength of the ordinary culture and contains 35 per cent glycerin.

*Firm E* recommends a 50 per cent solution of Koch's old tuberculin. This corresponds with the recommendations of Hutyrá and Marek, Joseph and others. This firm writes that they also dispense a purified tuberculin of strength equivalent to the above for intradermal use. They are not able to state the method of the preparation of this tuberculin, as it is manufactured in a foreign laboratory.

*Firm F* recommends tuberculin especially prepared for the intradermal testing of cattle. This firm could not give any particulars regarding the contents of the tuberculin or its method of preparation, as it was manufactured in a foreign laboratory.

In view of the diversity of tuberculins recommended for the intradermal test, the following information concerning the relative merits of alcoholic precipitated tuberculin versus Koch's old tuberculin is of interest:

*Alcoholic Precipitated Tuberculin.*—Using alcoholic precipitated tuberculin in solutions varying from  $\frac{1}{2}$  to 50 per cent, we have tested by the intradermal method 2312 cattle, 1190 of which reacted with clear and positive results. In one instance where dry alcoholic precipitated tuberculin was purchased from a certain laboratory and used intradermally on tuberculous cattle in 10 per cent solutions it gave very weak or negative results. Tests made with "purified tuberculin" of foreign manufacture and unknown strength have also given unsatisfactory results. We are now making tests on guinea-pigs to show the comparative toxicity of tuberculins from various manufacturers, the preliminary results of which indicate that tuberculins sold in this country vary considerably in strength. Government supervision and standardization of biological products, especially tuberculin, is greatly needed. When a potent precipitated tuberculin is not available it is preferable to Koch's Old Tuberculin for intradermal use. Our reasons for preferring the precipitated tuberculin to dilutions of Koch's Old Tuberculin are as follows:

In thirty-two of our intradermal tests with Koch's Old Tuberculin, the results were checked by injecting the opposite subcaudal fold with  $\frac{1}{5}$  cc. of physiological salt solution, containing 5 per cent glycerin and  $\frac{1}{2}$  per cent phenol, a new clean syringe being used. The swellings produced by the glycerin in two cases were persistent enough to be confusing. We then undertook extensive trials concerning the effect of solutions of glycerin, phenol and trikresol when injected intradermally and found that the glycerin in solutions of 5 per cent or stronger would frequently produce swellings large enough to be confusing when injected in  $\frac{1}{5}$  cc. doses. The trikresol and phenol in  $\frac{1}{2}$  per cent solutions on intradermal injection did not produce swellings.

We also tried the effect of injecting 10 per cent solutions of the precipitates derived by treating concentrated glycerin bouillon with alcohol in a manner similar to that used in producing precipitated tuberculin. These did not produce swellings large enough to be confused with an intradermal reaction.

Our observations in this respect corroborate the observations of Vallee, Declaire and Herbert.<sup>48</sup> They state that many healthy cattle, subjected to the intradermal test according to the method of Moussu and Mantoux,<sup>35-37</sup> show in about 6 per cent of the cases an immediate local swelling, at periods of time varying from five to sixty minutes after injection. This swelling at the site of the injection is edematous in character and varies in size from the dimensions of a hazelnut to those of a plum. The swelling disappears quickly, rarely persisting more than twenty-four hours, differing in this respect from the specific reaction in tuberculous animals, which is usually more persistent. Some cases, however, in healthy cattle persist more than forty-eight hours. The above authorities assume that the swelling is brought about in part by the glycerin or by certain salts in the bouillon and that it may be in part a reaction to a traumatism. According to Mulford's Veterinary Bulletin, they recommend the use of  $\frac{1}{2}$  per cent precipitated tuberculin solution with  $\frac{1}{5}$  cc. doses.

Under certain conditions, when the intradermal is to be followed by a subcutaneous test, it might be desirable to use this weak solution of tuberculin, as there would be less likelihood of its having an inhibitory effect on the subsequent test. In fact, Hamman and Wolman claim there is reason to believe that the injection of  $\frac{1}{10}$  cc. doses of  $\frac{1}{2}$  per cent tuberculin in a human being may have a sensitizing effect which will render a subsequent test more effective. We have no evidence to show that this holds true for cattle.

Foth<sup>11</sup> states that the dose used by the French authors was too small to get the maximum number of local reactions. This is probably true, but from our experiments we believe that the quantity of liquid injected should not exceed  $\frac{1}{5}$  cc. and that it is better to increase the concentration of the tuberculin instead of increasing the quantity of the dose. Most authorities prefer to use Koch's old tuberculin for the intradermal test and we would be inclined to agree in this if it were not for its glycerin content.

*Koch's Old Tuberculin (Tuberculin O. T. Original).*—Hutyra and Marek<sup>14</sup> state that a 50 per cent solution of Koch's old tuberculin, when injected intradermally, produces good clear results in both positive and negative cases. Several veterinarians who have had extensive experience in the use of tuberculin inform me that the ordinary B. A. I. tuberculin, undiluted, in doses of three to five drops, has given excellent results and that from a long series of tests in actual practice they do not believe it necessary to use purified tuberculin.

Joseph<sup>16</sup> has stated that not over  $\frac{1}{5}$  cc. should be injected, and we believe from our work that his suggestions should be followed, provided a strong solution of tuberculin is used. Joseph used  $\frac{1}{20}$  cc. of Koch's old tuberculin diluted with an equal amount of physiological salt solution, which would make a solution containing 25 per cent glycerin.

Using Koch's old tuberculin according to the method of Moussu and Mantoux,<sup>37</sup> we have tested by the intradermal method 1942 cattle, 477 of which reacted with clear and positive results. We have concluded, however, after due experimentation and consideration of the recommendations of the investigators and firms above mentioned, that a strong solution of alcoholic precipitated tuberculin is preferable to Koch's old tuberculin for the intradermal test in cattle.

The summary given in Table 1 is of interest in considering the relative merits of the various kinds and strengths of tuberculin recommended for the intradermal method.

In addition to the tests mentioned in Table 1, we have experimented with aqueous glycerin free suspensions of finely ground tubercle bacilli, also with suspension of whole tubercle bacilli which had been killed by heat, and with *phymatin*, a German preparation of unknown composition highly recommended for the ophthalmic and intradermal tests.

A summary of the autopsies on tested cattle is given in Table 2.

TABLE 1

## RESULTS FROM THE INTRADERMAL USE OF VARIOUS SOLUTIONS OF TUBERCULIN

Kind of tuberculin	Dose	Total number of cows tested	Total number of reactions	Conclusions regarding the tuberculin
Koch's old tuberculin dilution 1 to 10 of physiological salt solution.	$\frac{1}{10}$ to $\frac{1}{5}$ cc.	1942	477	Fairly satisfactory. Produces distinct local reaction swellings, but the glycerin which it contains causes a swelling immediately after injection which occasionally persists long enough in healthy cattle to be confusing. Thermal reactions also occur with this dosage.
Concentrated tuberculin equal in strength to a 70 per cent solution of Koch's old tuberculin.	$\frac{1}{10}$ to $\frac{1}{5}$ cc.	15	11	The tuberculin contains 35 per cent glycerin. The glycerin content is objectionable.
Koch's old tuberculin undiluted.	$\frac{1}{10}$ cc.	24	0	Used on 24 head of non-reacting cattle. The swellings due to the glycerin in some cases were four inches in diameter and persisted over 48 hours.
Alcoholic precipitated tuberculin 10 per cent solution (100 milligrams per cc. of physiological salt solution).	$\frac{1}{20}$ to $\frac{1}{5}$ cc.	201	48	This kind of tuberculin in strengths of 5 to 50 per cent is very satisfactory. It produces pronounced local and thermal reactions when injected intradermally in $\frac{1}{10}$ cc. doses. No local swellings occur in non-tuberculous cattle.
Alcoholic precipitated tuberculin 5 per cent solution (50 milligrams per cc.).	$\frac{1}{10}$ to $\frac{1}{5}$ cc.	937	630	
Alcoholic precipitated tuberculin 2 per cent solution (20 milligrams per cc.).	$\frac{1}{5}$ cc.	1055	479	Fairly satisfactory. Produces thermal as well as local reactions when injected intradermally. A stronger solution is better.
Alcoholic precipitated tuberculin $\frac{1}{2}$ per cent solution (5 milligrams per cc.).	$\frac{1}{5}$ cc.	119	33	Too weak except when the cattle are to be retested with stronger tuberculin. This strength of tuberculin produces moderate local and thermal reactions when injected intradermally.
A tuberculin of foreign manufacture recommended for intradermal use. Strength unknown.	$\frac{1}{5}$ cc.	17	4	The firm selling this tuberculin was unable to state its strength, saying it was manufactured in a foreign country. The autopsies proved this tuberculin unsatisfactory.
Total tests.		4310	1682	Autopsies were made in 341 cases. See notes following.
Subtracting for retests.		521	136	
Number of cattle tested.		3789	1546	



## AUTOPSY NOTES

Herd No. 1.—Fifteen cattle, which showed physical signs of tuberculosis, were injected with  $\frac{1}{2}$  cc. of a 10 per cent dilution of Koch's old tuberculin, and fourteen reacted. One week later they were tested by the subcutaneous method and all reacted. On autopsy they were found to be tuberculous. (See Table 6.)

Herd No. 3.—In testing a herd of 209 cattle, using the intradermal and subcutaneous methods simultaneously, one cow reacted to the subcutaneous test and another to the intradermal test. On autopsy these two were found tuberculous. (See cases 9 and 11, p. 132; also Table 6.)

Herd No. 6.—Two cattle that reacted locally to an intradermal injection of  $\frac{1}{2}$  cc. of a 10 per cent dilution of Koch's old tuberculin failed to react to subcutaneous tests applied three to thirty days later. They were found tuberculous on autopsy. (See cases 3 and 4, p. 130.)

Herd No. 8.—One hundred and seventy-two cattle, when tested after the method of Moussu, gave 124 reactions. Only two of these were autopsied, and both were found tuberculous. In addition, we observed thirty cows in this herd that evidenced physical signs of tuberculosis, several having symptoms of udder lesions. All of the physical cases reacted. (See Table 6, herd 8.)

Herd No. 9.—A certified herd of twenty-five cattle which had been tested semi-annually by the subcutaneous method and found free from reactions for five years was tested by the method of Moussu and one cow reacted. On autopsy she was found tuberculous. (For detailed description of this, see cow 1, p. 129.)

Herd No. 10.—Four out of eleven local reactors to concentrated tuberculin, containing 35 per cent glycerin, injected intradermally, failed later to react subcutaneously. Three of these were autopsied and tuberculous lesions found in two. It is not known whether the failure to find lesions in the third was due to the fact that only a hasty autopsy was made, or to the fact that the local swelling on this cow was due to the glycerin and not to a reaction. (See cases 2, 5, and 6, p. 130.)

Herd No. 13.—Thirty-five cattle tested intradermally with 2 per cent precipitated tuberculin gave fourteen reactions. Four of these were autopsied and all four contained tuberculous lesions.

Herd No. 24.—Thirty-six cattle were tested after the method of Moussu. One reacted. The entire thirty-six were autopsied and no tuberculous lesions were found. It is not known whether the failure to find lesions in the one showing the local swelling was due to the fact that only a hasty autopsy could be made or to the fact that the local swelling was caused by the glycerin and was not a reaction.

Herd No. 25.—Seventeen cattle were tested intradermally with a tuberculin of foreign manufacture. Four of them reacted. The entire seventeen were autopsied. Lesions could be found in only three of these. Two cattle which failed to react were found to have lesions. Obviously the tuberculin was defective.

Herd No. 29.—Eight hundred and eighteen cattle belonging to Dairy Company A were tested intradermally, using 5 per cent precipitated tuberculin, and 585 cattle reacted. There has been opportunity to autopsy only seven of these reactors. All but one of them showed tuberculous lesions. In addition, eight animals presumably physical cases of tuberculosis were found in the dairy, all of which reacted to the intradermal test.

Herd No. 30-35.—Two thousand one hundred and eighty-four cattle, belonging to Company B, were tested by the intradermal method, using various strengths of tuberculin, and 775 reacted. There has been an opportunity to autopsy thirty-two of the reactors, tuberculous lesions being found in all but one of these. Seven of the non-reactors were autopsied and all but one were found free from tuberculous lesions. The lesions in the tuberculous non-reacting animal were very extensive and the failure of the animal to react can be explained by the well-known fact that cattle having advanced tuberculosis frequently are not sensitive to tuberculin.

*Miscellaneous Autopsies.*—At various abattoirs in the San Francisco Bay region we have applied the intradermal test to 212 cattle and two or three days later have examined them on the killing floor. Various kinds of tuberculin were used. The results of these tests, together with the autopsies of cattle from the above mentioned herds are summarized in the following table:

TABLE 2  
RESULTS OF INTRADERMAL TESTS AND AUTOPSIES

Kind of Tuberculin	Cattle in which tuberculous lesions were found			Cattle in which no tuberculous lesions were found		
	Number tested	Number reacted	Per cent reacted	Number tested	Number reacted	Per cent reacted
10 per cent Koch's Old .....	27	25	92.6	36*	1	2.8
25 to 100 per cent Koch's Old	2	2	100.0	9	2	22.2
½ to 2 per cent precipitated....	36	34	94.4	10	1	10.0
5 to 10 per cent precipitated....	46	44	95.6	93	5	5.3
50 per cent precipitated .....	5	5	100.0	24	2	8.3
Phymatin .....	13	13	100.0	23	1	4.3
Foreign purified tuberculin.....	5	3	60.0	12	1	8.3

\* These thirty-six cattle were from ranges in Nevada where tuberculosis is rare, but there is reason to believe that the swelling was not a reaction but due to the glycerin in the tuberculin.

† Possibly not reactions. The swellings may have been due to glycerin in the tuberculin.



TABLE 3

INTRADERMAL TESTS WITH KOCH'S OLD TUBERCULIN IN 10 PER CENT SOLUTIONS AND PRECIPITATED TUBERCULIN  
IN 5 TO 10 PER CENT SOLUTIONS. RESULTS AND AUTOPSIES

Herd No.	Cow No.	Kind of tuberculin	Test result	Autopsy result	Physical condition and symptoms	Tuberculosis Lesions								
						Cervical glands	Thoracic glands	Lungs	Pleura	Liver	Uterus or Ovaries	Other abdominal lesions	Udder	Other lesions
1	1	10% O. T. ....	+	+	Bad, Lung symptoms .....	....	xxx	xxx	x	....	....	x	....	....
	2	10% O. T. ....	+	+	Poor, Jaw abscess .....	xxx	....	....	....	....	....	....	....	....
	3	10% O. T. ....	+	+	Emaciated, Dyspnea .....	x	xx	xxx	....	....	....	x	....	....
	4	10% O. T. ....	+	+	Fair, Lung symptoms .....	....	x	xx	x	....	....	....	....	x
	5	10% O. T. ....	+	+	Poor, Cough .....	....	x	xx	....	x	....	x	....	x
	6	10% O. T. ....	—	+	Emaciated, Cough .....	x	xx	xxx	xx	....	....	....	....	x
	7	10% O. T. ....	+	+	Poor, Lung symptoms .....	x	xx	xx	x	x	....	x	....	xx
	8	10% O. T. ....	+	+	Fair, Lung symptoms .....	....	x	x	x	....	....	x	....	....
	9	10% O. T. ....	+	+	Poor, Lung symptoms .....	....	x	xx	....	xx	....	x	....	....
	10	10% O. T. ....	+	+	Fair, No symptoms .....	....	x	xx	....	....	....	....	....	....
	11	10% O. T. ....	+	+	Good, No symptoms .....	x	....	xx	....	....	....	....	....	....
	12	10% O. T. ....	+	+	Good, No symptoms .....	....	x	x	....	....	....	....	....	....
	13	10% O. T. ....	+	+	Fair, No symptoms .....	....	x	x	....	....	....	....	....	....
	14	10% O. T. ....	+	+	Fair, No symptoms .....	....	....	....	....	....	....	x	....	....
	15	10% O. T. ....	+	+	Fair, No symptoms .....	x	x	xx	....	....	....	....	....	....
3	1	10% O. T. ....	+	+	Good, No symptoms .....	....	x	....	....	....	....	x	....	....
	2	10% O. T. ....	—	+	Good, No symptoms .....	x	x	....	....	x	....	....	....	....

TABLE 3—Continued

Herd No.	Cow No.	Kind of tuberculin	Test result	Autopsy result	Physical condition and symptoms	Tuberculosis Lesions								
						Cervical glands	Thoracic glands	Lungs	Pleura	Liver	Uterus or Ovaries	Other abdominal lesions	Udder	Other lesions
6	1	10% O. T. ....	+	+	Fair, No symptoms .....	.....	×	.....	.....	.....	.....	.....	.....	.....
	2	10% O. T. ....	+	+	Fair, No symptoms .....	.....	×	.....	.....	.....	.....	.....	.....	.....
	3	10% O. T. ....	+	+	Fair, No symptoms .....	.....	×	×	.....	.....	.....	.....	.....	.....
8	1	10% O. T. ....	+	+	Fair, No symptoms .....	.....	×	×	.....	.....	.....	.....	.....	.....
	2	10% O. T. ....	+	+	Poor, Lung symptoms .....	.....	×	×	.....	.....	.....	×	.....	.....
9	1	10% O. T. ....	+	+	Good, No symptoms .....	×	.....	.....	.....	.....	.....	×	.....	.....
24	1	10% O. T. ....	+	—	Excellent, No symptoms .....	No lesions found.								
Miscellaneous	1	10% O. T. ....	+	+	Thin, Lung symptoms .....	.....	×	×	×	×	.....	×	.....	.....
	2	10% O. T. ....	+	+	Emaciated, Lung symptoms	.....	xxx	xxx	×	.....	.....	.....	.....	×
	3	10% O. T. ....	+	+	Emaciated, Lung symptoms	.....	xx	xx	.....	.....	.....	.....	.....	.....
	4	10% O. T. ....	+	+	Emaciated, Lung symptoms	.....	×	xx	×	×	.....	×	.....	×
29	1	5% precipitated	+	+		*								
	2	5% precipitated	+	+		*								
	3	5% precipitated	+	+		*								
	4	5% precipitated	+	+		*								
	5	5% precipitated	+	+		*								
	6	5% precipitated	+	+		*								
	7	5% precipitated	+	—		*								
						No lesions found.								

\* Autopsy notes lost.

TABLE 3—Continued

Herd No.	Cow No.	Kind of tuberculin	Test result	Autopsy result	Physical condition and symptoms	Tuberculosis Lesions								
						Cervical glands	Thoracic glands	Lungs	Pleura	Liver	Uterus or Ovaries	Other abdominal lesions	Udder	Others
30-35	1	5% precipitated	+	+	Udder tuberculosis .....	xx	x	x	.....	.....	.....	.....	xx	.....
	2	5% precipitated	+	+	Thin, Lung symptoms .....	x	xx	xx	x	.....	.....	xx	.....	.....
	3	5% precipitated	+	+	Emaciated, Dyspnea .....	xx	.....	.....	.....	.....	.....	.....	.....	.....
	4	5% precipitated	+	+	Thin, Lung symptoms .....	xx	xx	xx	x	.....	.....	x	.....	.....
	5	5% precipitated	+	+	Emaciated, Lung symptoms .....	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	.....
	6	5% precipitated	+	+	Emaciated, Lung symptoms .....	.....	xx	xxx	xx	.....	.....	x	.....	.....
	7	5% precipitated	+	+	Thin, Lung symptoms .....	.....	xxx	.....	xx	.....	.....	x	.....	.....
	8	5% precipitated	-	+	Emaciated .....	.....	xxx	xxx	xxx	xxx	.....	.....	.....	.....
	9	5% precipitated	+	+	Fair, No symptoms .....	.....	x	.....	.....	.....	.....	x	.....	.....
	10	5% precipitated	+	+	Fair, No symptoms .....	.....	x	.....	.....	.....	.....	.....	.....	.....
11	5% precipitated	+	+	Fair, No symptoms .....	.....	x	x	.....	.....	.....	x	.....	.....	
12	5% precipitated	+	+	Fair, No symptoms .....	.....	xx	.....	.....	.....	.....	.....	.....	.....	
13	5% precipitated	+	+	Fair, No symptoms .....	.....	x	.....	.....	.....	.....	x	.....	.....	
14	5% precipitated	+	+	Fair, No symptoms .....	.....	x	.....	.....	.....	.....	.....	.....	.....	
15	5% precipitated	+	+	Fair, No symptoms .....	x	x	x	.....	.....	.....	x	.....	.....	
16	5% precipitated	+	+	Fair, No symptoms .....	.....	x	x	.....	.....	.....	x	.....	.....	
17	5% precipitated	+	+	Fair, No symptoms .....	.....	x	.....	.....	.....	.....	.....	.....	.....	
18	5% precipitated	+	+	Fair, No symptoms .....	.....	x	x	.....	x	x	x	.....	.....	
19	5% precipitated	+	+	Fair, No symptoms .....	.....	x	x	x	.....	.....	x	.....	.....	
20	5% precipitated	+	+	Fair, No symptoms .....	.....	x	xx	.....	.....	.....	x	.....	.....	
21	5% precipitated	+	+	Fair, No symptoms .....	.....	.....	.....	.....	.....	.....	x	.....	x	
22	5% precipitated	+	+	Fair, No symptoms .....	.....	x	.....	.....	.....	.....	.....	.....	.....	
23	5% precipitated	+	+	Fair, No symptoms .....	No lesions found.	.....	.....	.....	.....	.....	.....	.....	.....	
24	5% precipitated	+	+	Fair, No symptoms .....	.....	x	.....	.....	.....	.....	x	.....	.....	

TABLE 3—*Concluded*

Herd No.	Cow No.	Kind of tuberculin	Test result	Autopsy result	Physical condition and symptoms	Tuberculosis Lesions								
						Cervical glands	Thoracic glands	Lungs	Pleura	Liver	Uterus or Ovaries	Other abdominal lesions	Udder	Other lesions
30-35	25	5% precipitated	+	+	Fair, No symptoms	.....	xx	xx	.....	xx	.....	x	.....	.....
	26	5% precipitated	+	+	Fair, No symptoms	.....	xx	xx	.....	.....	.....	.....	.....	.....
	27	5% precipitated	+	+	Fair, No symptoms	.....	.....	.....	.....	.....	.....	x	.....	.....
	28	5% precipitated	+	+	Fair, No symptoms	.....	.....	.....	.....	.....	.....	x	.....	.....
	29	5% precipitated	+	+	Fair, No symptoms	.....	.....	xx	.....	.....	.....	.....	.....	.....
	30	5% precipitated	+	+	Fair, No symptoms	.....	x	xx	.....	.....	.....	x	.....	.....
	31	5% precipitated	+	+	Fair, No symptoms	.....	x	.....	.....	.....	.....	x	.....	.....
	32	5% precipitated	+	+	Fair, No symptoms	.....	.....	x	.....	.....	.....	.....	.....	.....
	33	5% precipitated	+	+	Fair, No symptoms	.....	.....	.....	.....	.....	.....	x	.....	.....
Miscellaneous	1	10% precipitated	+	+	Poor, Lung symptoms	.....	xx	xxx	xx	.....	xx	xx	x	xx
	2	10% precipitated	+	+	Fair, No symptoms	.....	x	.....	.....	.....	.....	.....	.....	.....
	3	10% precipitated	+	+	Good, No symptoms	.....	xx	.....	.....	.....	.....	.....	.....	.....
	4	10% precipitated	+	+	Good, No symptoms	.....	.....	.....	.....	xx	.....	xx	.....	.....
	5	10% precipitated	+	+	Good, No symptoms	.....	.....	.....	.....	.....	.....	.....	.....	x
	6	10% precipitated	+	—	Good, No symptoms	No lesions found.	.....	.....	.....	.....	.....	.....	.....	.....
	7	10% precipitated	+	+	Poor, No symptoms	.....	x	x	.....	.....	.....	x	.....	.....
	8	10% precipitated	+	—	Fair, No symptoms	No lesions found.	.....	.....	.....	.....	.....	.....	.....	.....
	9	10% precipitated	—	+	Fair, No symptoms	.....	xx	xx	x	x	.....	xx	.....	.....
	10	10% precipitated	+	—	Fair, No symptoms	No lesions found.	.....	.....	.....	.....	.....	.....	.....	.....
	11	10% precipitated	+	+	Fair, No symptoms	.....	x	xx	.....	xx	.....	xx	.....	.....

The above table does not include the 123 non-reacting cattle in which no tuberculous lesions were found.

The autopsy totals of cattle tested intradermally with 10 per cent Koch's Old Tuberculin or 5 to 10 per cent alcoholic precipitated tuberculin are:

	Number	Per cent
Number of reacting cattle in which tuberculous lesions were found .....	69	94.5
Number of reacting cattle in which no tuberculous lesions were found .....	4	5.5
Total for reacting cattle .....	73	100.0
Number of non-reacting cattle in which no tuberculous lesions were found .....	123	95.3
Number of non-reacting cattle in which tuberculous lesions were found .....	6	4.7
Total for non-reacting cattle .....	129	100.0

#### THE LOCAL REACTION TO THE INTRADERMAL INJECTION IN CATTLE

A positive intradermal reaction is indicated by a thickening of the subcaudal fold or by the appearance at the point of injection of a characteristic sensitive swelling varying in size from that of a small pea to that of an orange. The swelling may be either soft and oedematous or hard and inflamed. A recognizable reaction may be present on the sixth hour, but generally the reaction is first clear about the twelfth hour and continues to increase in size until the forty-eighth hour. In some instances we have observed that the early local reactions disappear before the forty-eighth hour, while in others delayed reactions have been noted which did not appear until the nineteenth hour. In order to be certain of every case, observations must be taken at least twice, preferably about the thirty-sixth and seventy-second hours. If only one observation can be made it should be on the seventy-second hour. *Small indurations at the point of inoculation about the size of the head of a parlor match frequently occur in normal non-reacting cattle, but anything larger than this which persists to the seventy-second hour should be considered a positive reaction.*

In recording the reactions, a convenient method for comparison is to describe the size of the swelling as pea size, hazelnut, walnut or hen's egg size. The exact size, if desired, may be measured by means of calipers such as are shown in plate 1, figure 3. Romer and Joseph<sup>43</sup>

consider that a thickening of the skin fold more than three millimeters larger than the thickness previous to injection should be considered a positive reaction. Cases in which the increase in thickness only amounts to three millimeters, they consider doubtful and subject to a retest. In our work, however, we have found the measurement of the swelling was not of much assistance. Experience and practice will enable the operator to judge of the size of the swellings without measuring, although for the sake of accurate records we have made a practice of taking careful measurements.

In judging a local swelling, the observer should depend more upon the shape, appearance, tenseness, sensitiveness and location with respect to the exact point of injection, than upon the actual measurements. Only experience can teach an operator how to be certain of a positive reaction when the local swelling is small. This is especially true when tuberculin containing glycerin has been used.

#### THE THERMAL REACTION TO THE INTRADERMAL INJECTION IN CATTLE

Moussu and Mantoux<sup>37</sup> state that no thermal reaction follows their method of performing the test. On the contrary, we have found that tuberculous cattle usually do show a marked temperature reaction when injected intradermally with the dosage and strength of tuberculin recommended by these investigators. Moussu and Mantoux used tuberculin brut (Koch's old tuberculin) diluted with ten volumes of physiological salt solution. Their dose was from  $\frac{1}{10}$  to  $\frac{1}{5}$  cc.

We have used this kind of tuberculin in testing 207 cattle when frequent temperature readings were taken after the intradermal injection. One hundred and twenty-four showed a local reaction swelling. In 101 of these 124 cases distinct temperature reactions also occurred. Three cows which showed a local, but no thermal reaction, were autopsied and found to be tuberculous. These cases are described in detail later.

The following table of twenty tests selected at random from the 101 cases mentioned above, shows the typical height of the thermal reaction to Moussu and Mantoux's method.



TABLE 4

TYPICAL CASES SELECTED TO SHOW THAT CATTLE REACT THERMALLY TO THE INTRADERMAL TEST.

Ear Tag No.	INTRADERMAL TEST RECORD										Size and description	SUBCUTANEOUS TEST RECORD																		
	Dose: 1/2 cc. of 10 % solution of Koch's Old Tuberculin											Dose: 2 cc. of 10% solution of Koch's Old Tuberculin																		
	TEMPERATURES					TEMPERATURES																								
Date: May 20, 1913					After Injection					Test Result	Before Injection					After Injection					Test Result									
Before Injection		6 hr.			9 hr.		12 hr.		14 hr.		16 hr.		18 hr.		20 hr.		8 hr.		10 hr.			12 hr.		14 hr.		16 hr.		18 hr.		20 hr.
31	101.1	101.2	102.4	104.8	106.6	105.0	103.1	101.0	101.4	+	40 mm, orange, hard	101.1	101.4	104.6	105.0	105.9	105.4	105.1	105.6	101.7	+									
32	100.8	101.8	102.8	104.0	103.6	103.3	102.0	101.4	100.8	+	20 mm, walnut, hard	101.0	101.0	102.4	103.6	103.9	104.0	102.4	101.6	-----	+									
36	101.6	103.2	103.9	104.6	104.5	105.4	104.5	104.2	100.1	+	13 mm, hazelnut, medium	101.2	102.1	104.2	105.4	105.2	104.2	103.4	104.4	104.6	+									
46	100.6	101.0	101.8	101.4	104.2	103.6	103.2	100.0	100.7	+	15 mm, walnut medium	100.8	101.4	101.5	104.6	104.8	103.2	101.8	103.5	102.8	+									
48	101.4	101.8	102.4	102.7	104.8	104.0	103.6	102.9	100.6	+	13 mm, hazelnut, medium	101.6	102.7	104.3	105.8	103.6	104.4	104.6	104.8	104.3	+									
56	101.2	101.8	103.2	106.3	105.6	105.6	104.0	102.2	101.8	+	20 mm, walnut, medium	101.1	101.8	103.7	104.9	105.8	103.5	103.2	102.6	-----	+									
66	100.9	102.0	103.0	105.1	106.0	103.0	102.0	101.4	100.4	+	20 mm, walnut, medium	100.5	101.6	103.1	105.0	105.6	104.7	104.0	104.8	103.6	+									
72	100.8	101.0	102.0	101.6	104.0	104.2	103.6	101.0	101.0	+	10 mm, pea, medium	100.6	101.8	101.4	102.2	104.4	104.3	103.5	104.7	102.6	+									
74	101.4	101.6	101.2	102.0	105.5	105.0	102.8	102.8	100.7	+	10 mm, walnut, hard	100.6	101.6	104.6	105.0	106.0	103.0	103.2	104.4	104.0	+									
76	101.0	101.4	102.6	105.2	105.2	105.2	104.0	103.0	101.4	+	20 mm, walnut, medium	101.4	101.6	105.8	105.6	103.8	102.8	102.6	103.0	-----	+									
80	101.0	102.0	104.0	103.2	102.2	101.8	100.8	100.6	100.4	+	10 mm, pea, medium	101.5	102.0	103.2	103.3	103.9	103.2	102.6	101.8	-----	+									
82	101.4	101.6	103.1	103.9	105.0	106.0	104.8	104.4	102.8	+	15 mm, walnut, medium	101.2	102.0	104.4	106.0	106.0	105.0	104.4	103.6	-----	+									
84	100.8	101.5	102.6	105.0	106.2	105.0	104.5	102.4	101.8	+	40 mm, orange, hard	100.8	101.7	106.1	106.2	104.0	103.0	102.3	102.0	-----	+									
95	100.6	101.4	104.0	105.5	105.6	104.6	103.8	103.1	102.8	+	25 mm, egg, medium	101.0	101.2	105.4	103.8	102.7	100.8	101.4	100.3	-----	+									
97	101.6	101.6	102.8	101.4	106.0	104.6	104.0	102.5	101.6	+	40 mm, orange, hard	100.4	101.7	104.9	104.1	104.2	103.2	101.8	103.6	103.5	+									
99	101.6	101.6	102.0	101.4	105.6	105.6	104.0	100.6	99.9	+	10 mm, pea, hard	101.2	101.2	101.1	132.8	104.2	104.8	103.8	102.4	-----	+									
103	101.8	102.5	103.1	103.3	106.6	106.2	105.8	105.4	100.1	+	20 mm, walnut medium	99.8	101.4	104.2	105.6	105.4	104.6	104.6	104.0	104.4	+									
113	101.3	102.0	102.5	103.2	105.4	105.1	103.6	102.4	102.0	+	13 mm, hazelnut medium	100.4	102.8	102.2	104.0	106.0	106.0	105.8	105.2	104.0	+									
114	100.2	101.4	104.4	104.2	102.4	102.6	102.6	102.0	101.7	+	20 mm, walnut, medium	101.2	101.4	102.6	104.6	105.9	103.7	102.1	102.2	-----	+									
										+	15 mm, walnut, medium	100.6	103.0	105.0	104.4	102.4	101.8	101.0	99.8	-----	+									

All animals in this table reacted to both tests.

In six herds where we have applied intradermal tests to cattle temperatures were taken at two-hour intervals from the eighth to the twentieth hour after the intradermal injection, and, in addition, in herd No. 8 from the second to the twentieth hour and in herd No. 20 from the eighth to the twenty-eighth hour.

TABLE 5

SHOWING TESTS IN SIX HERDS IN WHICH THERMAL REACTIONS USUALLY  
RESULTED FROM AN INTRADERMAL INJECTION

Herd No.	Total cows tested	Tuberculin used	Number of cattle that reacted locally	Number of cattle that reacted thermally to intradermal injection	Number of cattle that reacted locally but not thermally	Number of cattle that reacted thermally but not locally
8	172	10 per cent O. T.	124	102	22	0
12	21	2 per cent precipitated	1	1	0	0
11	25	10 per cent O. T.	1*	0	1	0
20	23	10 per cent precipitated	16	16	0	0
21	4	10 per cent precipitated	1	1	1	0
22	14	½ per cent precipitated	6	6	0	0
22†	14	10 per cent precipitated	4	4	1	0
Totals	273		153	130	25	0

\* See autopsy notes on cow Peggy, p. 129.

† Retest in three days.

The following table shows the results of tests made on six calves that had been injected with cultures of human tubercle bacilli six weeks previous for the purpose of immunizing them after the method of Pearson and Gilliland:

TABLE 6

RESULTS OF SUCCESSIVE INTRADERMAL TESTS WITH PRECIPITATED TUBERCULIN ON SIX VACCINATED CALVES

Test No. 1 on July 30, 1913. Dose: $\frac{1}{5}$ cc. of $\frac{1}{2}\%$ precipitated tuberculin solution.										Test No. 2 on August 5, 1913. Dose: $\frac{1}{10}$ cc. of $10\%$ precipitated tuberculin solution.											
THERMAL OBSERVATIONS										THERMAL OBSERVATIONS											
Temperature before injection		Temperature after injection				LOCAL OBSERVATIONS Size and description of swellings	Result	Temperature before injection		Temperature after injection				LOCAL OBSERVATIONS Size and description of swellings	Result	SUMMARY					
Min.	Max.	8th	12th	16th	20th			Min.	Max.	8th	12th	16th	20th			1st test	2nd test	Temperature	Swelling	Temperature	Swelling
1	102.0	102.8	102.7	104.5	106.2	104.6	+	55 mm., orange, hard	+	102.4	102.6	104.5	102.9	102.8	102.2	+	20 mm., walnut, soft	+	+	+	+
2	101.4	102.4	103.2	106.6	105.8	102.4	+	38 mm., orange, hard	+	101.6	102.6	105.6	103.2	101.8	101.8	+	15 mm., walnut, soft	+	+	+	+
3	101.4	103.6	103.6	105.6	105.6	101.8	+	20 mm., walnut, hard	+	102.2	102.4	104.2	102.6	102.9	102.4	+	20 mm., walnut, soft	+	+	+	+
4	101.6	103.2	103.2	105.4	105.4	103.4	+	30 mm., walnut, hard	+	101.8	103.2	103.6	102.4	102.6	102.4	+	16 mm., hazelnut, soft	+	+	+	+
5	101.4	102.6	102.0	102.0	103.6	102.2	+	12 mm., hazelnut, soft	+	102.0	102.6	103.6	101.4	101.1	101.8	+	12 mm., hazelnut, soft	+	+	+	+
6	101.4	101.6	100.6	101.0	102.6	103.0	+	18 mm., walnut, hard	+	101.0	102.0	100.8	101.2	101.0	101.6	—	20 mm., walnut, soft	+	+	—	+

It would appear from this table that the intradermal test has a slightly depressing effect on the reaction to an intradermal injection seven days later. The reaction swellings are uniformly smaller and the temperature does not rise so high after the second injection. The animals described in Table 4 were presumably healthy calves which on June 12, 1913, had been treated with cultures of human tubercle bacilli, strain Revenel M.

#### ACCURACY OF THE INTRADERMAL COMPARED WITH THE SUBCUTANEOUS METHOD

Investigators are so evenly divided on the question of which is the more accurate test that Table 7, showing comparative tests on one thousand cattle, including 326 reactors, is worthy of study in this connection. Moussu and Mantoux<sup>37</sup> in their original work compared the intradermal and subcutaneous tests on seventy reacting animals. They found that the two methods agreed in every case. In most instances they verified their findings by autopsy and concluded that the intradermal equals the subcutaneous test in accuracy. Vallee, Declaire and Herbert<sup>48</sup> tested 521 cattle by both methods. Four hundred and ninety-four reacted to the intradermal and 506 to the subcutaneous; that is twelve cattle with positive thermal reactions gave no intradermal reaction. Ward and Baker<sup>50-51</sup> tested 157 head, using the 10 per cent dilution of Koch's old tuberculin recommended by Moussu and Mantoux.<sup>37</sup> They verified their decisions by autopsy and concluded that the intradermal compares favorably in accuracy with the subcutaneous method. Zschocke<sup>55</sup> tested 300 cattle and found that 85 per cent of known tuberculous cattle reacted to the intradermal test. He considered the neck and tail injections of equal value. Longley<sup>23</sup> and McKenna<sup>26</sup> tested about 1500 cattle, using  $\frac{1}{2}$  per cent solutions of precipitated tuberculin, and decided that the intradermal test equals the subcutaneous in accuracy. Romer and Joseph,<sup>43</sup> using 50 per cent tuberculin solutions, found that one out of seventy-nine tuberculous animals failed to react to the intradermal test. Norgaard and Case,<sup>39</sup> after testing several thousand head, decided that the intradermal test was more satisfactory than the subcutaneous and stated that its substitution for the old method was fully warranted. Zwick and Tietze<sup>57</sup> admit no advantage for either the intradermal or

ophthalmic test and have not found them as accurate as the subcutaneous. Tietze<sup>47</sup> finds the intradermal more reliable than the ophthalmic. His conclusion is that the intradermal test is not as accurate as the subcutaneous. Lucky,<sup>24-25</sup> Sheldon,<sup>46</sup> Brown,<sup>2</sup> Kinsley and others in Missouri report, after having tested over thirty thousand head by the intradermal method, that this test is preferable under average conditions when applied by a skilled operator. Hutyra and Marek<sup>15</sup> state that it is desirable in practice where large herds are to be tested to apply a local test first, preferably the intradermal or ophthalmic test, and after the great majority of tuberculous animals have been recognized the remainder of the herd may be subjected to the subcutaneous test. Martin<sup>29</sup> recommends the intradermal test for general practice. Melvin,<sup>32</sup> of the United States Bureau of Animal Industry, reports that the ophthalmic and intradermal tests for the detection of tuberculosis in cattle have received attention. He says: "These methods thus far have not proved sufficiently superior to the subcutaneous injection of tuberculin to warrant their general application in practice, although they are still being tested whenever suitable opportunities are offered. A special preparation known as 'phymatin' has been used in the ophthalmic test and has given better results than the alcoholic-precipitated tuberculin. The ophthalmic and intradermal methods of applying the tuberculin test possess so many advantages, especially in the simplicity of reading the results, that the absolute acceptance of either one would be a great aid in the eradication of tuberculosis." Both methods obviate the laborious operation of taking temperatures repeatedly during the day after the injection and no preliminary temperatures are required. Littlejohn<sup>22</sup> advocates the application of several local tests simultaneously. Lignieres<sup>19</sup> recommends the simultaneous use of the intradermal, ophthalmic and subcutaneous tests. Foth<sup>11</sup> reports that he was able by the aid of the intradermal method to detect only about one-half the tuberculous individuals in Danish quarantine cattle. He states that the subcutaneous injection has a modifying effect on local tests applied simultaneously. Assmann<sup>1</sup> has observed in certain cases, where ophthalmic and thermal reactions occur at the same time, that the local reaction weakened as the thermal reaction gained strength.

The following table of one thousand comparative tests will be of interest to those who desire information concerning the comparative accuracy of the two methods. It will be noted that of the 326 cattle that reacted 35 failed to react to the intradermal method and 44 failed to react to the subcutaneous.



TABLE 7  
COMPARISON OF THE INTRADERMAL AND SUBCUTANEOUS TESTS, USING VARIOUS STRENGTHS OF TUBERCULIN FOR THE INTRADERMAL\*

Herd No.	Condition	Kind of tuberculin used for intradermal test	Total No. of cattle tested	No. of reactors to intradermal test	Questionable intradermal reactions	No. of reactors to subcutaneous *	Questionable subcutaneous reactions	No. reacting positively to intradermal and not to subcutaneous	No. reacting positively to subcutaneous and not to intradermal	Remarks
1	First time ever tested	10% Koch's Old	15	14	0	15	0	0	1	Subcutaneous test applied 7 days after intradermal. All the cattle were found tuberculous on autopsy.
2	First time ever tested	10% Koch's Old	37	3	0	2	0	1	0	Tests applied simultaneously.
3	Had been tested 6 mos. previous	10% Koch's Old	209	1	0	1	0	1	1	Tests applied simultaneously. For autopsy notes see cases Nos. 9, 11, pp. 132, 133.
4	Had been tested 6 mos. previous	10% Koch's Old	91	9	0	8	0	3	2	Tests applied simultaneously.
5	First time ever tested	10% Koch's Old	20	0	0	0	0	0	0	Tests applied simultaneously.
6	First time ever tested	10% Koch's Old	15	7	1	3	1	4	0	The subcutaneous was given 3 days after the intradermal. For autopsies see cases Nos. 3 and 4, p. 130.
7	First time ever tested	10% Koch's Old	21	1	0	1	0	0	0	Tests applied simultaneously.
8	First time ever tested	10% Koch's Old	172	124	1	124	3	10	10	Subcutaneous was given 10 days after the intradermal.
9	Tested every 6 mos. for years	10% Koch's Old	25	1	0	0	0	1	0	Tests given simultaneously. For autopsy see case No. 1.
10	First time ever tested	35% Koch's Old	15	11	2	7	0	4	0	Subcutaneous given 3 days after intradermal. 35% Koch's Old contains too much glycerin. For autopsies see cases Nos. 2, 5 and 6, p. 130.

\* For the subcutaneous test 2 to 4 cc. of Koch's Old Tuberculin 10 per cent was used. Temperatures after injection were taken at two hour intervals from the eighth to the twentieth hour.



TABLE 7—Continued

Herd No.	Condition	Kind of tuberculin used for intradermal test	Total No. of cattle tested	No. of reactors to intradermal reactions	Questionable intradermal reactions	No. of reactors to subcutaneous *	Questionable subcutaneous reactions	No. reacting positively to intradermal and not to subcutaneous	No. reacting positively to subcutaneous and not to intradermal	Remarks
11	First time ever tested	½ % precipitated	22	9	0	12	0	0	3	Tests given simultaneously.
12	First time ever tested	½ % precipitated	21	7	0	8	0	0	1	Tests given simultaneously. (½ % tuberculin is too weak.)
12	Tested 6 weeks previous	2% precipitated	21	8	0	8	0	0	0	Tests given simultaneously.
13	First time ever tested	2% precipitated	35	14	4	13	3	2	1	Subcutaneous given 3 days after intradermal. For autopsies see cases Nos. 7, 8 and 10.
14	"Plugging" suspected	5% precipitated	16	1	1	2	2	0	1	Tests given simultaneously.
15	First time ever tested	5% precipitated	3	1	0	1	0	0	0	Intradermal given 15 days after subcutaneous.
16	First time ever tested	5% precipitated	86	43	0	38	0	11	6	Tests given simultaneously.
17	Had been recently tested several times	10% precipitated	20	8	2	9	1	1	2	No subcutaneous injection was made. The thermal reactions resulted entirely from the intradermal injections.
18	Tested 3 mos. previous	10% precipitated	23	17	1	15	0	3	1	
19	Tested 6 mos. previous	10% precipitated	133	12	0	15	4	3	6	The subcutaneous test was given 20 days after the intradermal.
Totals			1000	291	12	282	14	44	35	

Total reactors to all tests, 326.

\* For the subcutaneous test 2 to 4 cc. of Koch's Old Tuberculin 10 per cent was used. Temperatures after injection were taken at two hour intervals from the eighth to the twentieth hour.

In herds 6, 10 and 13 it is unfair to judge the results from the subcutaneous test without considering the possibility of a modifying effect from the intradermal test just previous. We believe that the above results should be interpreted as indicating that the two tests are about equal in accuracy.

The results reported in tables A, B, and C are all by European observers and the proportion of reacting animals in which no lesions were found is much larger than has been reported by inspectors in the United States. As we do not know under what conditions these tests were performed no comparisons can be made with the results which we have obtained.

Klimmer and Wolff-Eisner<sup>17</sup> in their handbook of Serumtherapie and Serumdiagnosis have published the following tables which are of interest in a consideration of the intradermal method. It is possible that some of the figures in these tables were misprints, as the additions and percentages do not check in all cases.

TABLE A  
INTRADERMAL TESTS

Veterinarian	Kind of tuberculin	Tuberculous animals			Animals in which no lesions were found on slaughter		
		No. tested	Reacted No.	Per cent	No. tested	Reacted No.	Per cent
Assman <sup>1</sup>	Phymatin	40	29	72	11	5	45
Foth <sup>11</sup>	Bovotuberkulol 25%	11	5	45	4	0	0
	Bovotuberkulol 50%	15	7	48	20	1	5
	Old tuberculin (Höchst) 100%	15	8	52	45	13	29
Zschocke <sup>55</sup>	Phymatin 20%	46	39	84	53	11	21
	Phymatin 50%	40	23	57	21	1	5
	Phymatin (on swine)	14	13	93	82	1	1
	Bovotuberkulol 20%	16	9	56	21	7	33
	Bovotuberkulol (on swine)	1	1	100	14	0	0
	Tuberkulin (Marburg) 20%	4	1	25	9	0	0
	Tuberkulin (Marburg) 50%	18	9	50	23	7	30
	Tuberkulin (on swine)	1	1	100	23	0	0
Martin <sup>29</sup>	Tuberkulin (on swine) 50%	10	10	100	93	0	0
	Tuberkulin (on cattle) 50%	33	33	100	17	1	6
Joseph <sup>16</sup>	Tuberkulin (Marburg)	77	76	98	49	2	4.1
Total cattle .....		315	249	79	252	47	19
Total swine .....		26	25	96	212	2	1

TABLE B

RESULTS OF VARIOUS FORMS OF THE TUBERCULIN TEST ON TUBERCULOUS CATTLE

A positive cutaneous	reaction occurred in	39 out of 141 tuberculous cattle or 28%
A positive dermal	reaction occurred in	38 out of 143 tuberculous cattle or 27%
A positive intradermal	reaction occurred in	249 out of 315 tuberculous cattle or 79%
A positive vaginal	reaction occurred in	51 out of 66 tuberculous cattle or 77%

TABLE C

RESULTS OF VARIOUS FORMS OF THE TUBERCULIN TEST ON CATTLE IN WHICH NO LESIONS WERE FOUND ON SLAUGHTER

A positive cutaneous	reaction occurred in	6 out of 125 cattle or 5%
A positive dermal	reaction occurred in	3 out of 65 cattle or 5%
A positive intradermal	reaction occurred in	47 out of 252 cattle or 19%
A positive vaginal	reaction occurred in	3 out of 11 cattle or 27%

## RESULTS OF RETESTS

On retesting by any form of the tuberculin test, a badly infected herd, from which all the reacting cattle have been removed, it will usually be found that from 1 to 30 per cent will react. This is not necessarily due to the failure of the previous test, because the cattle may in the meantime have become infected from contaminated pastures or other sources.

Another reason for the reaction of such cattle on retest is the fact that cattle often do not react during the incubation of tuberculosis or while the disease is latent and the lesions encapsulated. In a badly infected herd many animals may be so recently infected that sensitiveness to tuberculin has not developed. In tuberculosis this period of incubation is variable. In some cases the disease probably begins to progress on the day of infection; in others the germs may be lodged in the tissues for a long time before lesions actually develop.

The arrest of the disease and encapsulation of the lesions is of frequent occurrence in the tuberculous cattle. This accounts for the fact that cows which have reacted occasionally fail to react when subsequently tested. Later the disease may start up and then, on a third test, the animals may again react. The commission appointed by the American Veterinary Medical Association have recognized these facts in their recommendation that herds over 50 per cent of which give positive reactions be handled, as far as eradication of the disease is concerned, as though all the herd had reacted.

The well-known fact that animals in advanced stages of the disease frequently do not react applies equally to the intradermal and subcutaneous methods, but from our observations we think the former will detect more arrested or latent cases. The results in retesting compare favorably with those we have obtained with the subcutaneous method under similar conditions. The following data showing the results of retests is typical of what is to be expected.

## TESTS FOR DAIRY COMPANY B

- Sept. and Oct., 1913. Tested by the intradermal method, 2184 cattle, 775 reacted, 35.5 per cent. (Three hundred of the non-reactors were segregated on one ranch and four months later 228 of them were retested; 176 of the reacting cattle were also retested in four months.)
- Feb., 1913. Retested 228 non-reacting cattle, 46 reacted, 20.2 per cent.
- Feb., 1913. Retested 176 reacting cattle, 173 reacted, 98.3 per cent.

Following are the results of frequent subcutaneous tests in three dairies:

## DAIRY No. 1.

	No. of cows tested	No. con- demned	Per cent
January 5, 1905 .....	77	14	18.3
March 12, 1906 .....	62	2	3.2
September 29, 1906 .....	83	6	7.2
September 14, 1907 .....	144	32	22.2
January 29, 1908 .....	130	22	16.9
August 12, 1908 .....	128	4	3.1
April and September, 1909 .....	125	2	1.6
January 30, 1910 .....	114	7	6.1
August 1, 1910 .....	99	4	4.0
March 21, 1911 .....	118	4	3.4
Purchases to June, 1910 .....	342	84	24.5
Purchases since June, 1910 .....	87	3	3.4
Total number of cattle tested .....			506
Total number condemned .....			184
Per cent condemned .....			36.4
Total number of tests, including retests .....			1,509

## DAIRY No. 2

	No. of cows tested	No. con- demned	Per cent
February, 1908 .....	196	110	56.1
February, 1909 .....	189	13	6.9
November, 1909 .....	176	37	21.0
June, 1910 .....	246	18	7.3
February 15, 1911 .....	208	19	9.1
Purchases to June, 1910 .....	385	135	35.1
Purchases since June, 1910 .....	14	.....	.....
Total number of cows tested .....			595
Total number condemned .....			332
Per cent condemned .....			55.8
Total number of tests, including retests .....			1,414

## DAIRY No. 3

	No. of cows tested	No. con- demned	Per cent
November, 1908 .....	70	12	17.1
April, 1909 .....	57	9	15.8
November, 1909 .....	73	4	5.5
May, 1910 .....	138	2	1.5
November, 1910 .....	198	6	3.0
Purchases to June, 1910 .....	203	5	2.5
Purchases since June, 1910 .....	320	8	2.5
Total number of cows tested .....			593
Total number condemned .....			46
Per cent condemned .....			7.8
Total number of tests, including retests .....			1,059

In the last three dairies the reacting cattle were at once removed and the barns were frequently disinfected. Additions to the herd were non-reactors selected chiefly from infected herds. In the case of the cattle belonging to Dairy Company B the non-reacting cattle were separated from the reacting, but thorough disinfection was impossible. Since the conditions in the various dairies differed materially, no comparisons between the intradermal and subcutaneous tests are justified.

DESCRIPTION OF CASES WHICH REACTED TO THE INTRADERMAL  
BUT DID NOT REACT TO THE SUBCUTANEOUS TEST

The following detailed history of ten cows which reacted to the intradermal but not to the subcutaneous test is submitted as proof that cattle, having latent, slight or inactive lesions of tuberculosis, may fail to react to the subcutaneous test, but may react to the intradermal.

Except when otherwise stated, all tests in these cases were made with Koch's old tuberculin, dilution 1 to 10 of physiological salt solution. The dose for the intradermal tests was  $\frac{1}{5}$  cc. The dose for the subcutaneous tests varied from 2 to 4 cc.

**COW 1.** Peggy. Grade Holstein. Good physical condition throughout the time she was under observation.

Oct. 10, 1911. Tested by subcutaneous method. **No reaction.**

Mar. 2, 1912. Tested simultaneously by the subcutaneous and intradermal methods. **No thermal reaction to the subcutaneous. Strong reaction to the intradermal.**

Apr. 2, 1912. Tested by the intradermal method. **Strong reaction.**

July 20, 1912. Tested by the subcutaneous method. **No reaction.**

Oct. 4, 1912. Tested by the subcutaneous method. **No reaction.**

Jan. 1, 1913. Tested by the intradermal method. **Strong reaction.**

Mar. 8, 1913. Tested simultaneously by the subcutaneous and intradermal methods. **No thermal reaction to the subcutaneous. Slight reaction to the intradermal.**

July 10, 1913. Tested by the intradermal method, using  $\frac{1}{5}$  cc. of 10 per cent solution of purified tuberculin. **Slight reaction.**

July 12, 1913. This cow was slaughtered for beef under municipal inspection. One of the retropharyngeal lymph nodes contained a tuberculous nodule five millimeters in diameter with a wall one millimeter thick composed of greyish connective tissue-like substance. In the center was a small amount of caseous yellow material, partially calcified. In one of the mesenteric lymph nodes a lesion very similar in size and appearance was found. No other lesions were discovered. A guinea pig was inoculated with the tissue and on autopsy August 13, 1913, was found to be extensively tuberculous.

**COW 2.** Ear tag No. 1649. Fair physical condition.

May 6, 1913. Tested by intradermal method. 35 per cent O. T. tuberculin. **Positive reaction.**

May 8, 1913. Tested by subcutaneous method. **No reaction.**

June 6, 1913. Tested by ophthalmic method. **No reaction.**

June 23, 1913. Tested by ophthalmic method. 2 per cent purified tuberculin. **No reaction.**

June 26, 1913. Tested by the intradermal method.  $\frac{1}{2}$  per cent purified tuberculin. **Positive reaction.**



**COW 2—(Continued).**

- June 29, 1913. Tested by the intradermal method. 2 per cent purified tuberculin. **Positive reaction.**
- July 10, 1913. Tested by the intradermal method. 10 per cent purified tuberculin. **Positive reaction.**
- July 12, 1913. Killed for beef under federal inspection. Four tuberculous nodules similar in appearance to those in Cow 1 (Peggy) were found in the bronchial lymph nodes. No other tuberculous lesions were discovered.

**COW 3.** Ear tag No. 6. Aged, fair physical condition.

- May 6, 1913. Tested by intradermal method. **Positive reaction.**
- May 8, 1913. Tested by subcutaneous method. **No reaction.**
- June 6, 1913. Tested by the ophthalmic method. **Positive reaction.**
- June 23, 1913. Tested by ophthalmic method. 2 per cent purified tuberculin. **No reaction.**
- June 26, 1913. Tested by intradermal method.  $\frac{1}{2}$  per cent purified tuberculin. **Positive reaction.**
- June 29, 1913. Tested by intradermal method. 2 per cent purified tuberculin. **No reaction.**
- July 10, 1913. Tested by intradermal method. 10 per cent purified tuberculin. **Positive reaction.**
- July 12, 1913. Killed for beef under municipal inspection. The anterior and posterior mediastinal lymph nodes were enlarged to three times normal size and contained dry calcareous and fibrous tuberculous lesions apparently inactive and of long standing. No other lesions were found.

**COW 4.** Ear tag No. 1219.

- May 6, 1913. Tested by intradermal method. **Positive reaction.**
- May 8, 1913. Tested by subcutaneous method. **No reaction.**
- June 6, 1913. Tested by ophthalmic method. **Positive reaction.**
- June 23, 1913. Tested by ophthalmic method. 2 per cent purified tuberculin. **No reaction.**
- June 26, 1913. Tested by intradermal method.  $\frac{1}{2}$  per cent purified tuberculin. **Positive reaction.**
- June 29, 1913. Tested by intradermal method. 2 per cent purified tuberculin. **Positive reaction.**
- July 10, 1913. Tested by intradermal method. 10 per cent purified tuberculin. **Positive reaction.**
- July 12, 1913. Killed for beef under municipal inspection. Acute lesions of tuberculosis involving both anterior lobes of the lungs and the mediastinal and bronchial lymph nodes.

**COW 5.** Ear tag No. 1206. Good physical condition.

- May 6, 1913. Tested by intradermal method. 35 per cent O. T. tuberculin. **Strong positive reaction.**
- May 8, 1913. Tested by subcutaneous method. **No reaction.**
- June 6, 1913. Tested by ophthalmic method. **No reaction.**
- June 23, 1913. Tested by ophthalmic method. 2 per cent purified tuberculin. **No reaction.**

**COW 5—(Continued).**

- June 26, 1913. Tested by intradermal method.  $\frac{1}{2}$  per cent purified tuberculin.  
**No reaction.**
- June 29, 1913. Tested by intradermal method. 2 per cent purified tuberculin.  
**Positive reaction.**
- July 10, 1913. Tested by intradermal method. 10 per cent purified tuberculin.  
**Positive reaction.**
- July 12, 1913. Killed for beef under municipal inspection. No tuberculous lesions were found. An unusually careful search was made from a meat inspection standpoint, but it was impracticable to strip the meat from the bones and search the interior of the bones and joints and certain other places where small tuberculous lesions might have existed.

**COW 6. Ear tag No. 1218.**

- May 6, 1913. Tested by intradermal method. 35 per cent O. T. tuberculin.  
**Positive reaction.**
- May 8, 1913. Tested by subcutaneous method. **No reaction.**
- June 6, 1913. Tested by ophthalmic method. **No reaction.**
- June 23, 1913. Tested by ophthalmic method. 2 per cent purified tuberculin.  
**No reaction.**
- June 26, 1913. Tested by intradermal method.  $\frac{1}{2}$  per cent purified tuberculin.  
**Positive reaction.**
- June 29, 1913. Tested by intradermal method. 2 per cent purified tuberculin.  
**Positive reaction.**
- July 10, 1913. Tested by intradermal method. 10 per cent purified tuberculin.  
**Positive reaction.**
- July 12, 1913. Killed for beef under municipal inspection. Soft caseous tuberculous lesions in the liver and between the liver and the diaphragm. One mesenteric lymph node was caseous.

**COW 7. Ear tag No. 68. Fair physical condition.**

- May 6, 1913. Tested by intradermal method. 2 per cent purified tuberculin.  
**Positive reaction.**
- May 8, 1913. Tested by subcutaneous method. 2 per cent purified tuberculin.  
**No reaction.**
- June 6, 1913. Tested by ophthalmic method. 2 per cent purified tuberculin.  
**No reaction.**
- June 23, 1913. Tested by ophthalmic method. 2 per cent purified tuberculin.  
**No reaction.**
- June 26, 1913. Tested by intradermal method.  $\frac{1}{2}$  per cent purified tuberculin.  
**Positive reaction.**
- June 29, 1913. Tested by intradermal method. 2 per cent purified tuberculin.  
**Positive reaction.**
- July 10, 1913. Tested by intradermal method. 10 per cent purified tuberculin.  
**Positive reaction.**
- July 12, 1913. Killed for beef under federal inspection. Tuberculous lesions were present in the anterior lobes of both lungs and in the bronchial and retropharyngeal lymph nodes.

**COW 8.** Ear tag No. 1213.

- May 6, 1913. Tested by intradermal method. **Positive reaction.**  
May 8, 1913. Tested by subcutaneous method. **No reaction.**  
June 6, 1913. Tested by ophthalmic method. **Positive reaction.**  
June 23, 1913. Tested by ophthalmic method. 2 per cent purified tuberculin.  
**No reaction.**  
June 26, 1913. Tested by intradermal method.  $\frac{1}{2}$  per cent purified tuberculin.  
**Positive reaction.**  
June 29, 1913. Tested by intradermal method. 2 per cent purified tuberculin.  
**No reaction.**  
July 10, 1913. Tested by intradermal method. 10 per cent purified tuberculin.  
**Positive reaction.**  
July 12, 1913. Killed for beef under municipal inspection. Two small tuberculous nodules in the liver, each about 1 cm. in diameter, having firm fibrous walls 1 mm. in thickness. Contents yellow, firm, sprinkled with points of deeper yellow color, calcification was not present and the tissue was not entirely broken down.

**COW 9.** Ear tag No. 1638.

- Jan. 14, 1912. Tested by subcutaneous method. **No reaction.**  
June 6, 1912. Tested by subcutaneous method. **No reaction.**  
Jan. 3, 1913. Tested by subcutaneous method. **No reaction.**  
June 6, 1913. Tested by subcutaneous method. **No reaction.**  
Tested by intradermal method. **Strong reaction.**  
July 7, 1913. Autopsied. In the mediastinal lymph nodes were two tuberculous lesions each about two-thirds the size of the fist, encapsulated and partially liquified. In the mesenteric lymph node was a soft caseous tuberculous lesion two centimeters in diameter.

**COW 10.** Ear tag No. 1220. Fine physical condition throughout the tests.

- May 6, 1913. Tested by intradermal method. 2 per cent purified tuberculin.  
**Positive reaction.**  
May 8, 1913. Tested by subcutaneous method. **Positive reaction.**  
June 4, 1913. Tested by ophthalmic method. **Positive reaction.**  
Aug. 20, 1913. Tested simultaneously by the subcutaneous and intradermal methods. **Positive reaction to both tests.**  
Oct. 24, 1913. Tested by ophthalmic method. **Positive reaction.**  
Oct. 26, 1913. Tested by ophthalmic method. **Positive reaction.**  
Dec. 4, 1913. Tested simultaneously by the ophthalmic, subcutaneous and intradermal methods. **Positive reactions to the ophthalmic and intradermal tests, no reaction to the subcutaneous method.**  
Dec. 5, 1913. Autopsied. Two very small tuberculous lesions in the lymph nodes on rumen.

Zwick and Tietze<sup>27</sup> state that the intradermal and ophthalmic tuberculin tests are of no value in distinguishing the progressive cases from those having arrested latent or healed lesions. We agree in this, in that we have not been able to apply in a practical way the

different forms of the tuberculin test to the classification of tuberculous cattle according to the extent or activity of their lesions. However, the cases which react locally to the intradermal test, but which fail to react thermally either to the intradermal or subcutaneous injections, have usually been found on autopsy to contain small encapsulated lesions.

DISCUSSION OF CASES WHICH REACTED THERMALLY TO THE  
SUBCUTANEOUS BUT SHOWED NO LOCAL REACTION  
TO THE INTRADERMAL TEST

We have observed thirty-five cases which showed a thermal reaction to the subcutaneous method but no local reaction to the intradermal method. This should be considered in judging the comparative value of the intradermal test. Fourteen of the above mentioned thirty-five cows were tested by both methods on the same day. One of these was afterward autopsied and found to contain a few active tuberculous lesions. Following is her history:

**COW 11.** Ear tag No. 2187.

Jan. 5, 1913. Tested by subcutaneous method. **No reaction.**

June 27, 1913. Tested by subcutaneous method. **Positive reaction.**  
Tested by intradermal method. **No reaction.**

July 7, 1913. Autopsied. The lungs contained one tuberculous lesion the size of a half dollar, encapsulate with caseous center. One bronchial gland contained a calcified tuberculous lesion five millimeters in diameter. The liver contained two well-healed lesions, averaging one centimeter in diameter.

The failure of this and thirty-four other cows to react locally might be accounted for by the theory that a local reaction is weakened, or disappears, when it occurs simultaneously with a temperature reaction. The observations of Foth<sup>11</sup> and Assmann<sup>1</sup> already quoted are of interest in this connection. However, we have observed so many instances in which marked thermal and intradermal reactions occurred simultaneously from an intradermal injection that more evidence is desirable before accepting this theory. We are inclined to attribute the failure of the intradermal test to the well-supported theory that no form of the tuberculin test will detect every case of tuberculosis in cattle, and that the maximum number of reactors can only be determined by applying the various tests simultaneously, or the local tests first and following them by the subcutaneous method

after an interval of at least seven days. Assmann<sup>1</sup> has pointed out that the proportion of questionable, or faulty, diagnoses amount to over 13 per cent for the thermal, while he admits only 9 per cent of failures with the ophthalmic test.

Hastings<sup>14</sup> states that probably in 15 per cent of the animals examined in badly infected herds the subcutaneous test is in error. By far the greater number of errors is due to the non-reaction of tubercular animals. Both the thermal and intradermal tests occasionally fail to produce reactions in tuberculous cattle under circumstances that exclude an explanation on the ground that the disease was either latent, or so advanced that the cattle would not react. The possibility of the animals having been rendered insusceptible by previous injections of tuberculin can also be eliminated.

#### THE COMPARATIVE ADVANTAGES OF THE INTRADERMAL AND OPHTHALMIC METHODS IN TESTING CATTLE

Six state veterinarians have recently written us that they consider the intradermal method preferable to the ophthalmic. Hutyra and Marek<sup>15</sup> also indicate a preference for the intradermal over the ophthalmic. Our data on this point are rather meager. In tests on known tuberculous animals with 10 per cent solutions of Koch's old tuberculin our results were unsatisfactory, being negative in all instances. Five per cent solutions of precipitated tuberculin, however, proved very satisfactory. In 139 cattle known to be reactors to the subcutaneous or intradermal methods, when tested by the ophthalmic method, using this tuberculin, we produced pronounced conjunctival reactions in every case. In one instance a cow reacted to the ophthalmic test, but failed to react to either the intradermal or

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\* The ophthalmic tuberculin test as first applied to cattle in 1907 did not give very uniform results, probably because it was made either with a weak solution or with tuberculin diluted with irritating solutions of glycerin, trikresol, or phenol. Recent observations by Foth<sup>11</sup> and others have shown that 5 per cent solutions of dry tuberculin give very good results in this test.

*Technic of the Ophthalmic Test.*—An assistant should grasp the animal by the nose and turn the head at an angle so that the operator may drop one or two drops of the tuberculin directly upon the eyeball. A small hypodermic syringe is more suitable for this purpose than a medicine dropper. The closed eye is then slightly massaged for several seconds. In cattle the reaction usually sets in by the eighth hour. It manifests itself by a watering of the eye, reddening of the conjunctiva and dropsical swelling of the lid. Later a purulent exudate accumulates at the inner corner of the eye and usually runs down the face. This sometimes dries up and drops off in a few hours, so that the evidences of the reaction may entirely disappear in twenty-four hours, although occasionally they can be seen for several days. Although the results of the test are usually apparent by the twelfth hour, additional observations should be made on the eighteenth and twenty-fourth hours to detect any delayed reactions.



subcutaneous tests. On autopsy this cow proved tuberculous. In testing range cattle we have found it easier to apply the intradermal than the ophthalmic test. The eye test is more reliable than either the intradermal or subcutaneous methods for testing animals which have been recently injected with tuberculin. The test can be repeatedly applied without injury to the eye. Its frequent repetition does not injure its accuracy. When given simultaneously with, or subsequent to, either the intradermal or subcutaneous test it may occasionally assist in deciding doubtful reactions. Since the reaction usually occurs by the eighth hour, it is sometimes found preferable in veterinary practice to use this method when quick results are necessary. Our attempts to use the ophthalmic method in the routine testing of dairy cows have failed to give satisfaction. In one instance when the test was applied to a dairy of eighty-six cows, known to include at least forty reacting animals, the rain washed out or obscured all but one conjunctival reaction. In other instances we have observed that the exposure to bright sunshine and wind has caused equally unsatisfactory results. The test is only satisfactory when the cattle can be kept tied or confined in stanchions under shelter for a period of at least sixteen hours, except in the case of animals which show a reaction before that time. The cattle should be fed only on concentrates, or finely chopped hay. We have verified the observation of White and McCampbell that it is advisable not to feed long hay while the reaction is developing on account of the possibility of wiping off the exudate. Klimmer,<sup>17</sup> Assman,<sup>1</sup> Melvin,<sup>32</sup> Konge<sup>18</sup> and others have reported excellent results with the ophthalmic test when using a preparation known as phymatin. In testing twenty-three tuberculous cattle with this preparation we have obtained positive reactions in every case. Phymatin will also produce reactions when injected intradermally.

#### USE OF THE INTRADERMAL TUBERCULIN TEST ON SWINE

At the University of California Hog Serum Laboratory the intradermal test is used as a matter of routine on all hogs purchased. The test is very satisfactory, the reactions usually being larger in size than in cattle. A dose of at least  $\frac{1}{5}$  cc. should be used. We are undecided as to the best place to make the injection. Injections into the skin at the edge of the ear are very easily seen. However, when the injection is made at the base of the ear the swellings are often larger. Tuberculous hogs often show a thermal as well as a local reaction to  $\frac{1}{5}$  cc. doses of a 10 per cent solution of alcoholic precipitated tuberculin.

Following is a statement of certain tests made by Dr. J. F. Mitchell at the University of California Hog Serum Laboratory: Sixty-nine hogs were tested by the intradermal method, using 10 per cent Koch's Old Tuberculin, and fifteen reacted. Three days later the ophthalmic test was administered, using the same kind of tuberculin. No eye reactions occurred, except possibly in one case, which showed a very slight discharge from one eye. Twenty days later sixty-two of these hogs, including the fifteen reactors, were again tested by the intradermal method and twenty reacted. We used 2 per cent precipitated tuberculin on nineteen of these, including four that had previously reacted. Six reactions resulted with this tuberculin including the four hogs which had reacted at the previous test. Ten per cent Koch's Old Tuberculin was used on the remaining forty-three, including nine hogs that had reacted at the previous test, and fourteen of the forty-three reacted, including the nine that had previously reacted.

Fourteen days later eighteen of the reacting hogs were given the ophthalmic test, using 5 per cent precipitated tuberculin, and seven of these showed a very slight yellowish discharge in the eye injected, but not enough to be of any great diagnostic value. Nine of the reactors have been killed, and in one of them we failed to find lesions; the others were tuberculous. In only one case was the entire carcass condemned for tuberculosis.

#### CONDITIONS UNDER WHICH THE INTRADERMAL TEST IS PREFERABLE

Since the reaction to tuberculin when injected by the intradermal method depends on a swelling at the point of injection and not on thermal manifestations, it is to be preferred to the subcutaneous injection under all conditions that are liable to modify the tuberculin temperature curve. In testing wild range cattle, even if it is possible to restrain them, no reliance can be placed on the reaction as shown by the tuberculin temperature curve.

In calves under six months of age there is liable to be a difference of two or more degrees in the normal temperature on two consecutive days. The temperature of some cows is easily influenced by variations in the weather and also by changes in handling and feeding. We deem it advisable to test by the intradermal method if the temperature of the air where the cattle are confined exceeds 100 degrees F. or in cold weather if the animals will be compelled to stand in a draught. Cattle from a long journey by road or rail are apt to have an abnormal temperature.

If an animal reacts locally there is a record left at the point of injection in the form of a swelling, this swelling being discernible in some instances three weeks after injection.

The local intradermal reaction is not affected by advanced pregnancy, recent parturition or the period estrum. We hesitate to mention this as an advantage over the subcutaneous method, however, for in our experience in testing numerous cows in this condition we have not observed that the reliability of the subcutaneous test was affected.

Although we have reason to believe that the intradermal method modifies to some extent the results of a subsequently performed subcutaneous test, its effect is certainly not so pronounced as a previous subcutaneous test. When cattle are to be retested a weak solution ( $1\frac{1}{2}$  per cent precipitated tuberculin) can be used. We believe that the intradermal test can always be satisfactorily substituted for the subcutaneous by an experienced operator, provided a strong tuberculin (5 per cent or stronger solution of precipitated) is used for the intradermal injection and daily observations on the swellings are continued to the ninetieth hour. In fact, we are convinced that when performed in this way on large, badly infected herds more tubercular animals will be detected than when dependence is placed on temperature reaction alone.

It is reported that in certain pure bred herds where the owners permit prospective purchasers to apply the tuberculin test the animals sometimes receive several tests a year. The substitution of either the intradermal or ophthalmic test, preferably the latter, would be desirable in such herds, because these tests, not being so liable to render the infected animals insensitive to tuberculin, would have the advantage.

#### CONDITIONS UNDER WHICH THE INTRADERMAL TEST IS UNRELIABLE

Tuberculous cattle that have been recently injected with tuberculin frequently do not respond to the intradermal test. The method does not seem to have much advantage over the subcutaneous in this respect, and is inferior to the ophthalmic for retesting. Repeated intradermal tests render tuberculous cattle unresponsive to either the subcutaneous or intradermal methods.

In the hands of an inexperienced man the intradermal is not so reliable as the subcutaneous or ophthalmic methods. Even a graduate

veterinarian who has not received special instruction or at least perfected himself in the interpretation of the local swellings by several autopsies or observations of this test when administered in conjunction with the usual subcutaneous method, in our opinion is not capable of securing good results.

Since small doses are used in the intradermal method it is of the highest importance that the tuberculin be of a high standard of potency. It must be sterile and free from glycerin. It must be administered in a clean and skillful manner. A bungling operator, by introducing the needle several times may cause traumatic injuries which may have the appearance of reactions. Observations on the reactions should be continued until the seventy-second hour. Under no circumstances can this test be finished as quickly as the other methods. If pressed for time the veterinarian must depend on the ophthalmic and subcutaneous method. Dr. Law in his treatise on veterinary medicine, mentioned seventeen conditions under which he believes the subcutaneous method to be unreliable, but in most instances these do not apply to the intradermal method. In competent hands this method has the advantage over either the subcutaneous or ophthalmic under average California conditions.

Cattle in a very thin, starved condition are not suitable subjects for any form of the tuberculin test. In testing some dry dairy cows that had been turned out on scanty pasture, we observed that several known tuberculous animals failed to react to the subcutaneous test. The preliminary results of experiments which we are making on some stock indicated that during the winter, when the feed is poor and the cattle are somewhat reduced in flesh, their sensitiveness to tuberculin is not as pronounced as when they are in prime condition.

#### ANSWERS TO CERTAIN OBJECTIONS TO THE INTRADERMAL METHOD

An argument that has been advanced against the use of the intradermal method for official tuberculin testing is that tabulated records of the test results cannot be made. Would not a tabulation of the measurements of the swellings, together with notes on their appearance and sensitiveness, constitute an acceptable test record? Vallee<sup>49</sup> suggests that a series of observations be expressed graphically. An advantage of the intradermal test in this respect is that reacting swellings usually persist for several days and in some cases for weeks. The presence or absence of such a swelling in case of dispute would constitute evidence which would add to the value of the tuberculin test record. We have observed that some cattle which reacted to the



intradermal showed a return of the local reaction when retested by the subcutaneous method. Occasionally this recurring local reaction has exceeded the original in size. This return of the subcaudal swelling after the injection of tuberculin subcutaneously in the neck has been observed even after an interval between tests of three months.

Another objection is that it requires an expert and experienced man to use the intradermal method successfully. We admit that the test demands some experience, but have pointed out that any practitioner can gain that experience while applying the usual subcutaneous method. On the other hand, it is not a test which can be as readily used by the layman as the subcutaneous method. We mention this latter fact, knowing that it may appeal to many as a favorable argument, although it is to be regretted that there has not as yet been perfected a test so simple and accurate that any dairyman can use it on his own herd. We believe that the ophthalmic comes nearer to this than either the intradermal or subcutaneous method.

The very just objection is made that as yet we do not know whether the test is as accurate as the subcutaneous method. More data are needed on this point. Comparison should be made by autopsy, and I regret that in California we have been able to follow up with autopsies only 341 cases out of 4001 tested. To be of any value such autopsies should be made on the non-reacting as well as reacting animals. Most tests that have been recently made to determine the value of the intradermal have included the autopsy of both the reacting and non-reacting animals. The intradermal test is so easy to apply that it would be possible for federal or state officials to arrange with abattoirs to apply the test to thousands of head and follow them to the killing floor. Titze<sup>47</sup> has concluded after a study of the results of the intradermal and ophthalmic tests in Germany that these methods are not equal in accuracy to the subcutaneous. Quoting statistics of the U. S. Bureau of Animal Industry,<sup>31</sup> he assumes that it is possible to make a sure diagnosis by the subcutaneous method in more than 97 per cent of tuberculous cattle. Hastings<sup>14</sup> has pointed out the fallacy of such deductions. Although 98.93 per cent of the since no autopsies were made on them. We believe that the sub-under supervision had tuberculous lesions, it is not known what proportion of the 363,008 non-reacting cattle really had tuberculosis. 24,784 reacting cattle reported by Melvin<sup>31</sup> as having been autopsied cutaneous test as ordinarily performed on large numbers of cattle will fail to detect tuberculosis in at least 10 per cent of the occult cases. Wills<sup>54</sup> found with the subcutaneous test that over 20 per cent of the thermal reactions in seventy-two tuberculous cattle occurred



subsequent to the eighteenth hour after injection. Even if it is eventually found that the intradermal test fails to detect tuberculosis in 10 or 15 per cent of occult cases, we would still consider it as efficient as the subcutaneous method.

The strongest objection to the intradermal test seems to be that as yet a standard tuberculin for this test has not been perfected. With the six leading biological houses of this country each putting out a different kind of "intradermal tuberculin," some of which, to say the least, are unsuitable for the purpose, uniform results are impossible.

We have advocated in this paper the combination of the intradermal and ophthalmic with the subcutaneous method. The objection is that this requires too much time and work. This is the crux of the whole bovine tuberculosis situation. Such objections are made by practitioners who are willing to slight their work in order to obtain an easy fee. In case it is impossible to perform the subcutaneous test thoroughly, the intradermal should certainly be used. Schnürer<sup>45</sup> has pointed out that the combination of several tests increases the certainty of the results. The combined tests add accuracy by confirming the many questionable reactions that occur and by detecting tuberculous cows that react to but one test. At present it would be a backward step to abandon the subcutaneous method for official work. Combining the two tests gives time for physical examination, in which more thoroughness is sadly needed in this state.

#### SUMMARY

In all, 4926 intradermal tests have been made on 4001 cattle, 1614 of which reacted, and there has been an opportunity to check these results by autopsies in 341 cases and by the discovery of lesions on physical examination in 54 additional cases.

To 291 cattle that reacted to the intradermal test the subcutaneous test was subsequently or simultaneously applied, and 282 cattle reacted. Forty-four cattle with a positive intradermal reaction gave no thermal reaction to the subcutaneous test. It was possible to autopsy only ten of these which failed to react to the subcutaneous method, but in nine of the ten tuberculous lesions were found. Thirty-five cattle which failed to react to the intradermal test subsequently reacted to the subcutaneous test. Two of these cows which reacted to the subcutaneous method, but showed no swelling at the point of intradermal injection, were found on autopsy to be tuberculous.

Cattle which react to the intradermal test with local swelling usually have also a thermal reaction similar to that produced by the ordinary subcutaneous injection.

The intradermal test is unreliable when applied a few days after a subcutaneous injection. The time limits under which this holds true have not been determined.

The intradermal test appears to have some modifying effect on a subsequent test by either the intradermal or subcutaneous methods, but this is not so pronounced as that produced by the usual subcutaneous injection.

The thermal reaction which accompanies the intradermal injection usually subsides in twenty hours, but it seems desirable not to apply the subcutaneous test for several days after the intradermal. The longer the interval the better.

The subcaudal fold is a more suitable point for the intradermal injection than the neck.

Our results with Koch's Old Tuberculin in 10 per cent solutions and with alcoholic precipitated tuberculin in  $\frac{1}{2}$  per cent solutions compare favorably in accuracy with the usual subcutaneous method. A more satisfactory method, however, is to use  $\frac{1}{3}$  cc. intradermal doses of alcoholic precipitated tuberculin in 5 to 50 per cent solutions administered subcaudally, and 2 cc. doses of 10 per cent Koch's Old Tuberculin administered subcutaneously, and take temperatures as in the usual subcutaneous test. With this kind and strength of tuberculin the local reactions are pronounced and the thermal reactions are as characteristic as those that occur from subcutaneous injections alone.

The procedure in injecting cattle is not difficult, provided needles of the proper length are used. To obtain the best results the tuberculin must be injected into the lower layers of the skin or into the subdermal connective tissue. The application of strong disinfectants to the site of injection should be avoided. It requires considerable experience to correctly interpret the local swellings. This is especially true when tuberculin containing glycerin is used.

We do not advocate the substitution of the intradermal for the subcutaneous method by a veterinarian until he has become skilled in its use by practice and observation. The practitioner can easily accomplish this in the routine of his work by applying the two tests simultaneously. Such a procedure cannot injure the accepted subcutaneous method in any way, although it may modify the local intradermal swellings to some extent.

Our observation of the fact that cattle having small, latent, or arrested lesions will sometimes react to the intradermal and not to the

subcutaneous test suggests the possibility of differentiating between latent and acute infections by combining the two tests, but we have not been able to put this into practical use.

When 5 per cent solutions of potent precipitated tuberculin are used both the ophthalmic and intradermal methods equal the subcutaneous test in accuracy, but we have found the ophthalmic method unsuitable for use in routine testing under California conditions, except when the cattle can be kept indoors.

The intradermal method has proven very satisfactory in the out-of-door tuberculin testing frequently necessary in this state and, for the non-official testing of large numbers of cattle, is preferable to the subcutaneous method. It requires more experience to apply the test and interpret the intradermal reactions than is required in testing by the subcutaneous or ophthalmic methods.

Some tuberculous cattle react locally to the intradermal test, but fail to react thermally to the subcutaneous test, while others which react thermally fail to react locally. Obviously, then, it is necessary to use both tests in order to detect the maximum number of reacting cattle. By administering the ophthalmic, intradermal and subcutaneous tests simultaneously, a higher percentage of tuberculous cattle will be detected than if dependence is placed on one test alone.

The intradermal test is especially adapted to the testing of swine and has proven of value in the routine work of the California Hog Serum Laboratory.

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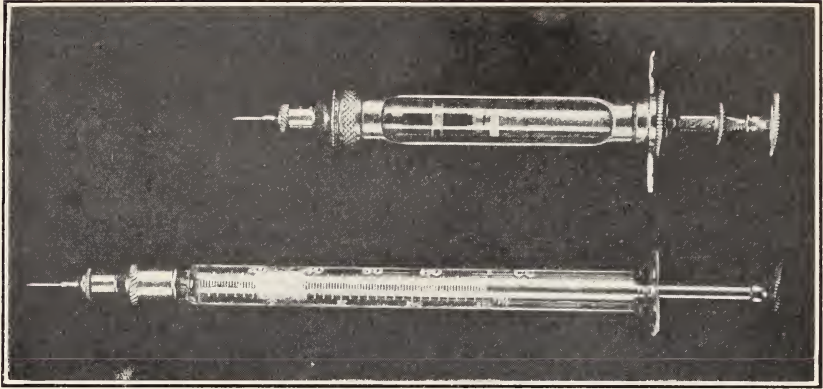
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C. M. H.

PLATE 1



FIGURES 1 AND 2

Either of the two syringes shown above is suitable for intradermal injecting. The shorter one is a dental syringe of 30 minims capacity, with a burr on the piston which can be used to regulate the dose. For intradermal work the needle point should be about one-quarter of an inch long.

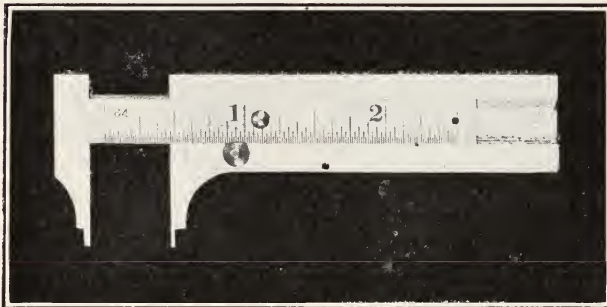


FIGURE 3

Calipers similar to those shown above are useful in determining the exact size reaction of swelling

## PLATE 2

In making an intradermal injection, one of the folds of skin beneath the base of the tail is grasped between the thumb and fingers and the needle inserted, slightly horizontally, into the thickness of the skin between the thumb and fingers. If the needle is properly placed the tuberculin can be felt in the deeper layers of the skin as it is injected, and after the removal of the

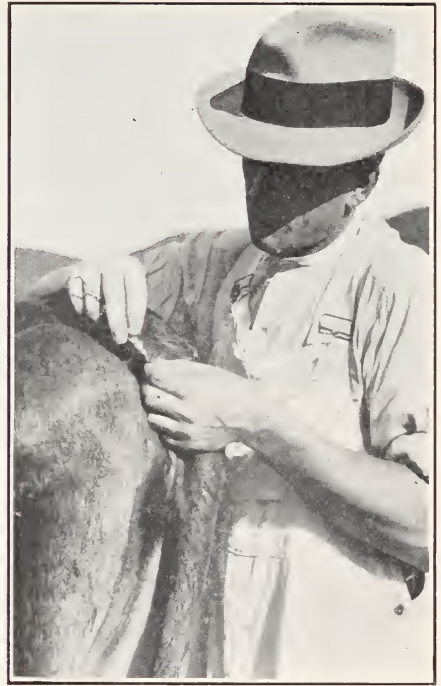


FIGURE 1



FIGURE 2

needle the tuberculin can be felt as a lump about the size of a small pea. The best reactions are obtained when the tuberculin is injected into the deeper layers of the skin. Inject deep enough so that the fluid does not swell out the skin like a blister. We consider Figure 1 a more convenient position for operating than Figure 2.

## PLATE 3

The figures in this plate show typical intradermal reactions. A reaction to the intradermal tests is indicated by a swelling or a thickening of the skin at the point of injection. The swelling may be either soft or pendulous, as in Figure 1, or hard and inflamed as in Figure 2.

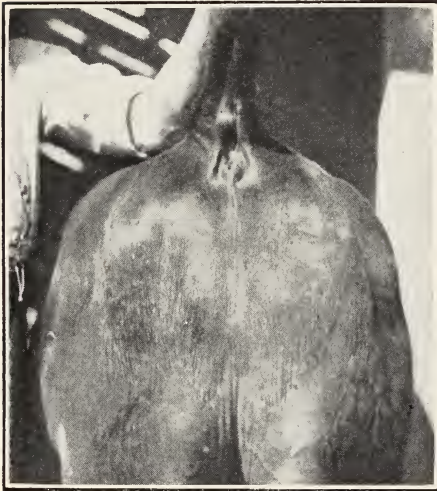


FIGURE 2

In some instances we have observed that the early local reactions disappear by the forty-eighth hour, while in other cows delayed reactions have been noted that did not appear until the ninetieth hour. In order to be certain of every case, observations should be taken about the thirtieth and seventieth hours after injection.



FIGURE 1

A recognizable reaction may be present at the sixth hour, but generally the reaction swelling is first clear about the twelfth hour and continues to increase in size until the forty-eighth hour.

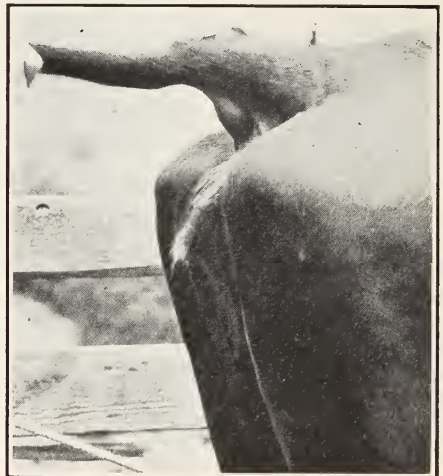


FIGURE 3



## PLATE 4



FIGURE 1

should depend on the shape, appearance, tenseness, sensitiveness and location with respect to the exact point of in-



FIGURE 3

The use of calipers to determine the exact size of the swellings is shown in the figures on this plate. However, in judging a local swelling to determine if it is a positive reaction to tuberculin, one

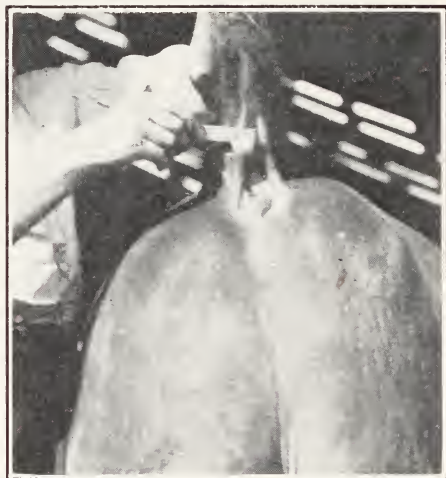


FIGURE 2

jection more than upon the actual measurements. Only experience can teach one how to be certain of a positive reaction when the local swelling is small. This is especially true when tuberculin containing glycerin is used.

## PLATE 5

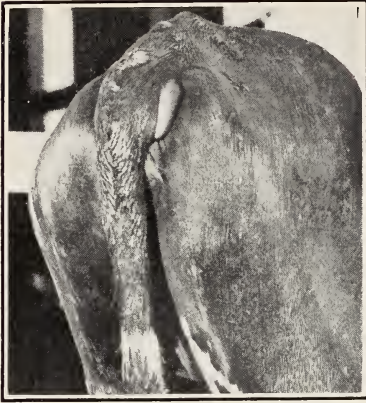


FIGURE 1

Figure 3 shows the swellings due to two injections with a six-day interval. The left subcaudal fold was injected July 30th and a local reaction the size of an orange

As shown in Figure 1 the intradermal reaction is sometimes so large that it can be seen without raising the tail.

Figure 2 shows an intradermal reaction that obliterates both subcaudal folds.

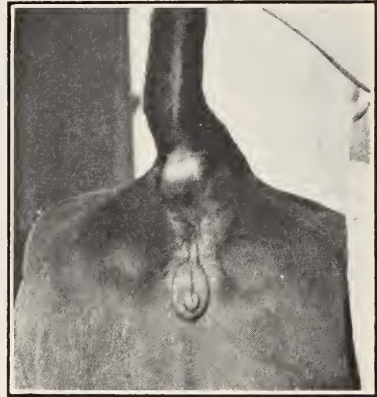


FIGURE 2

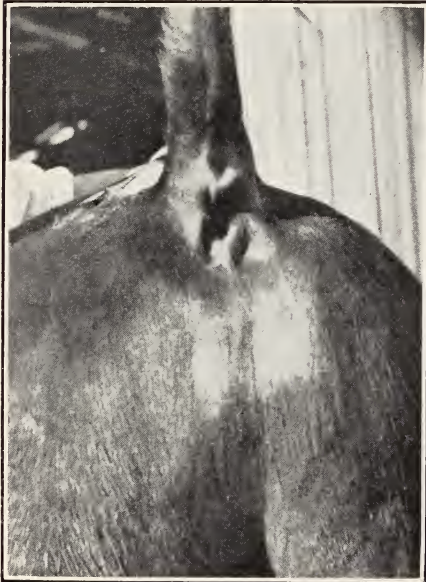


FIGURE 3

occurred, which persisted several weeks. The right fold was injected August 5th and resulted in a local reaction the size of a walnut. (See Animal 1, Table 6.) Thermal reactions usually accompany intradermal reactions.

One advantage of the intradermal reaction is that it often leaves a more or less permanent mark which can be used to identify the reacting animals for several weeks.

## PLATE 6

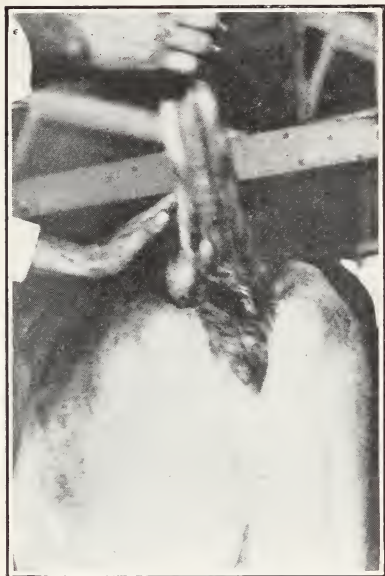


FIGURE 1

Figure 2 shows an intradermal reaction in a calf six weeks of age. The test is especially adapted to the testing of young calves and wild range cattle.



FIGURE 2

Figure 1 is not a cut of a reaction, but shows a swelling caused by the application of a strong solution of lysol. Irritating disinfectants should not be used before applying the intradermal test.



## PLATE 7



FIGURE 1

The ophthalmic or eye test with tuberculin may equal the other forms of tuberculin testing in accuracy, but under the average outdoor conditions of California is not as suitable as the intradermal test.

Figure 1 shows a mild ophthalmic reaction twelve hours after injection.



Figure 2 shows a severe ophthalmic reaction twelve hours after injection.

FIGURE 2

## PLATE 8



FIGURE 1



FIGURE 2

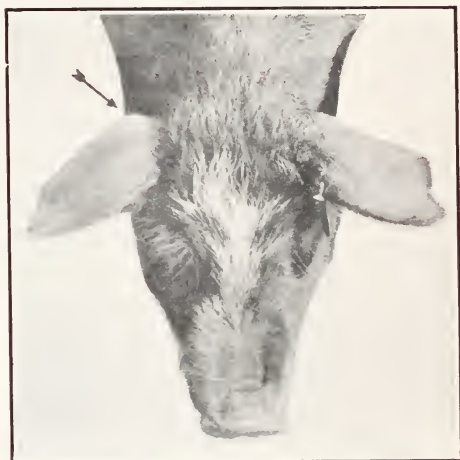


FIGURE 3

The intradermal test is especially adapted to the testing of swine. Injections may be made into the back of the ear near its base as in Figure 1, or into the edge of the ear as in Figure 2. The reaction swellings are similar to those in cattle (see Figure 3).



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